Histological Evaluation of local application of peripheral blood mesenchymal stem cells and platelet rich fibrin matrix /Bone morphogentic protein 9 (BMP9) on bone healing

By
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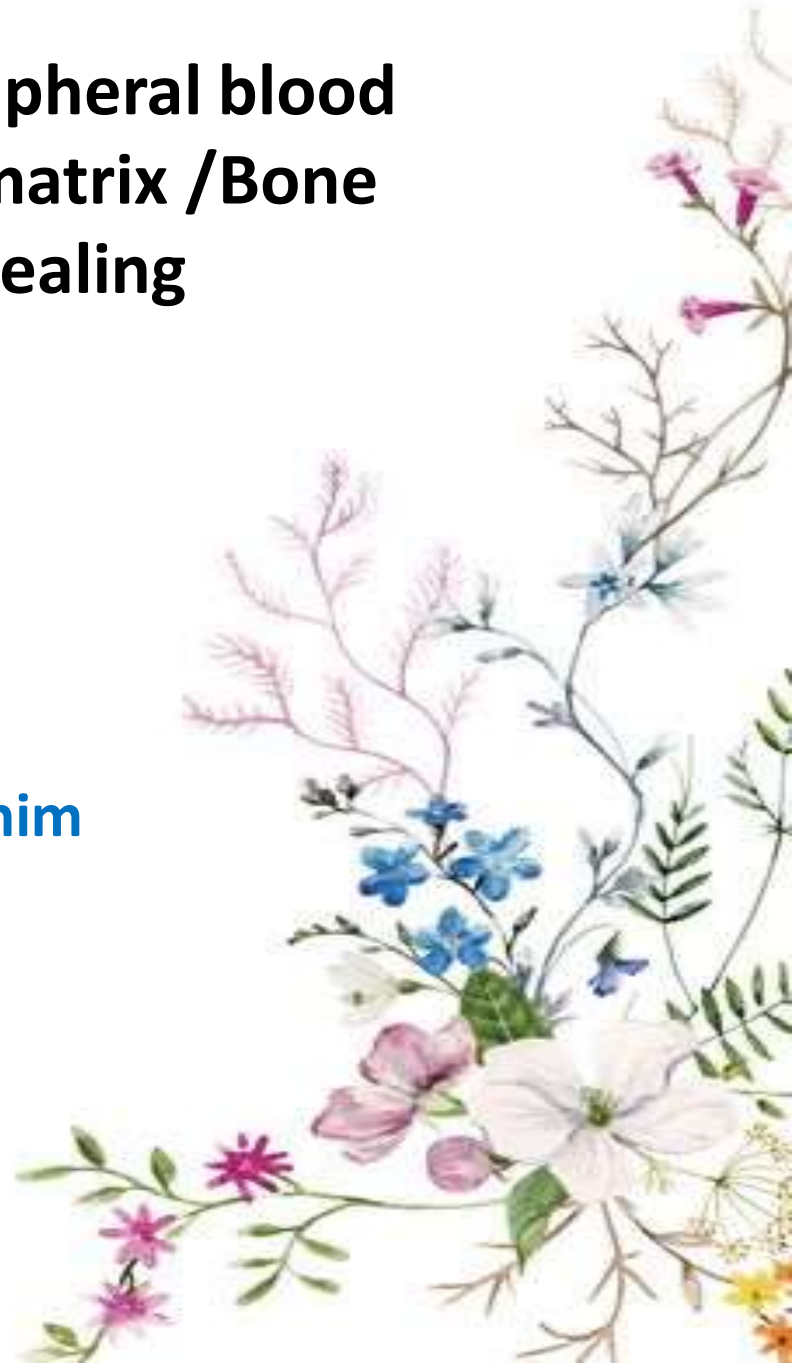
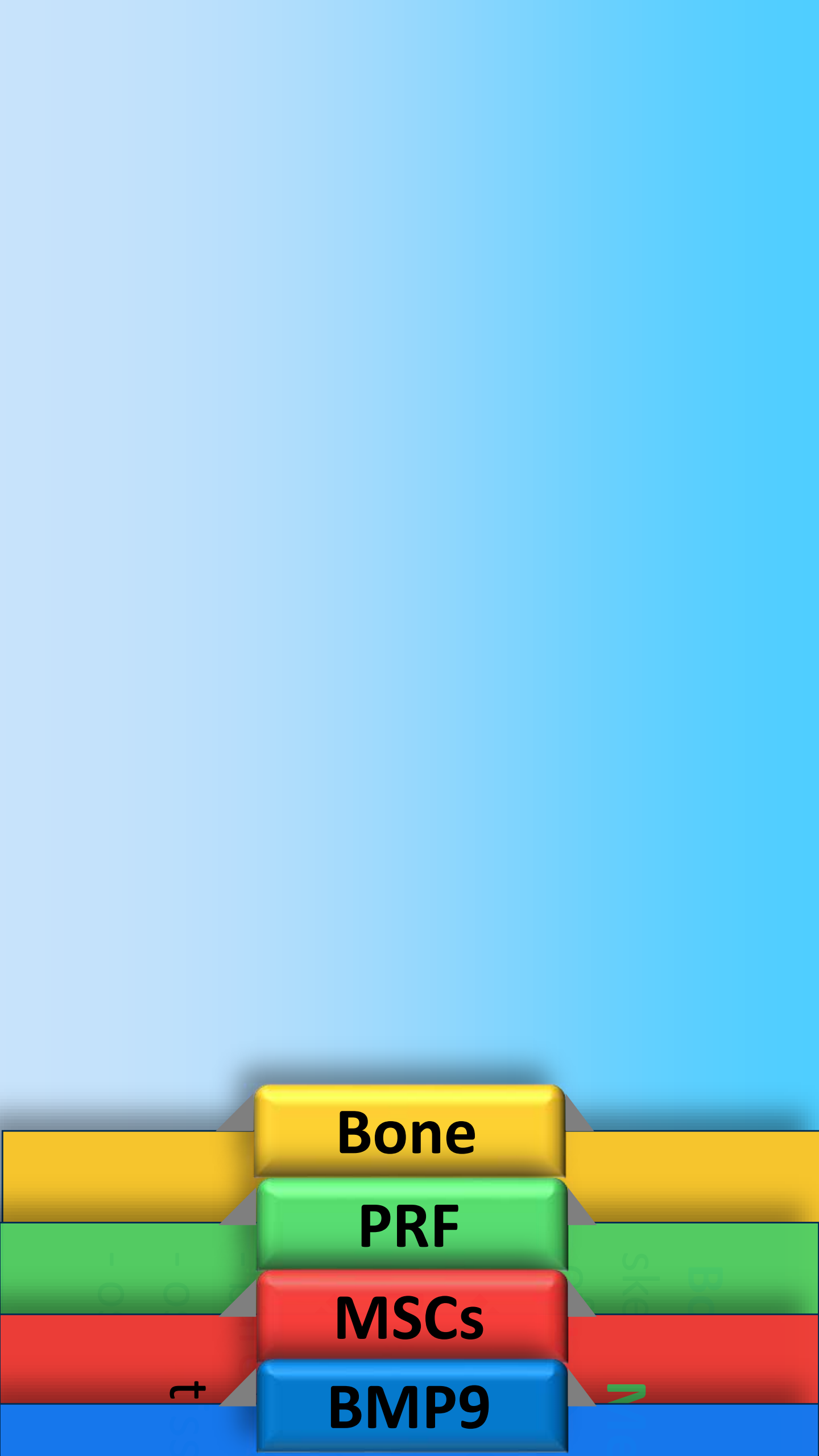


Introduction

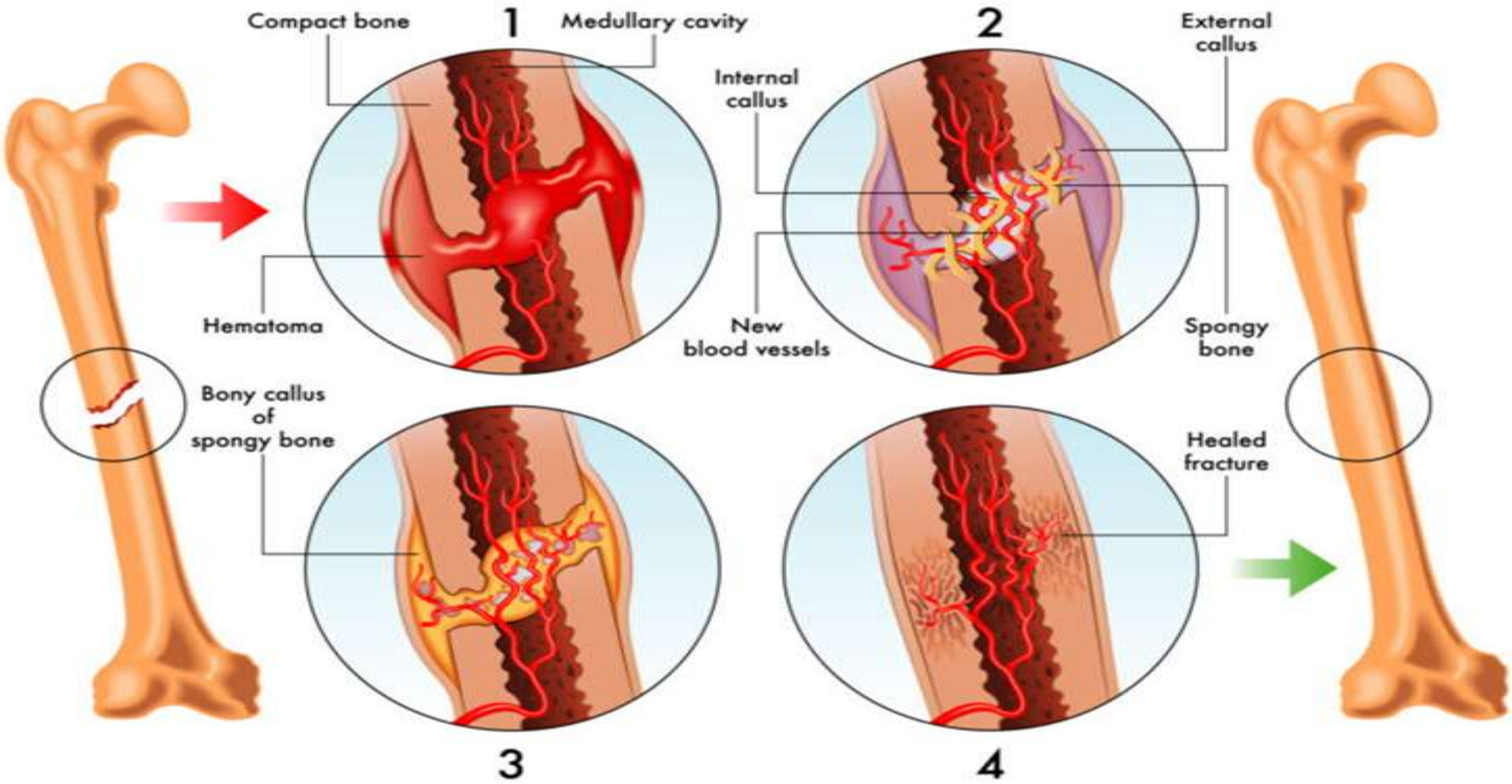

Bone
PRF
MSCs
BMP9

Objectives of the study

Study the effect of platelet rich fibrin matrix with stem cells, bone morphogenic protein 9 and their combination on bone healing by means of histological and histomorphometric analysis.

Literature Review

Bone healing: is a regenerative process in which bone is restored without scare tissue formation


Compact bone
1
Medullary cavity
Hematoma
Bony callus
of
spongy bone
3
Internal
callus
2
External
callus
New
blood vessels
Spongy
bone
Healed
fracture
4

Regeneration

Regeneration is an enigma by itself, So it is difficult

AN ENIGMA

Numerous procedures have been investigated in the past to try to promote soft and osseous tissue regeneration.

However

are highly

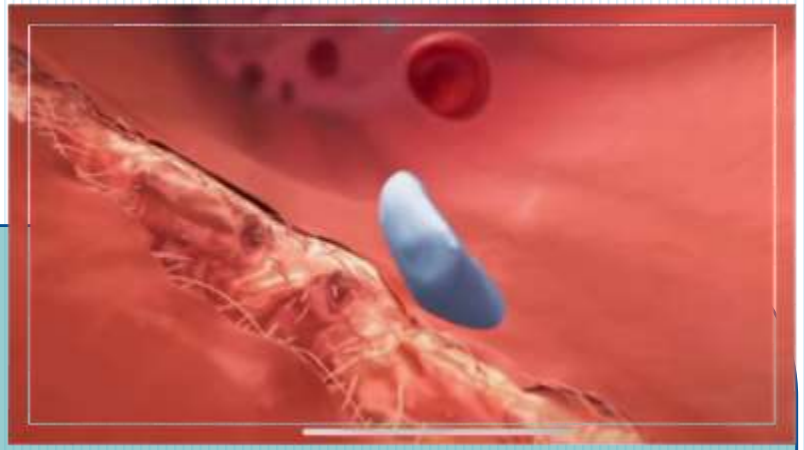
But the best
Biological solutions for Biological problem

-Tissue Engineering

-Application Of Biologics,

-Cell Based Techniques,

-Biomimetic Nanoscaffold Fabrication …..etc



Platelet Rich Fibrin Matrix (PRFM):
is a biological matrix of ECM protein + growth factors derived from peripheral blood.

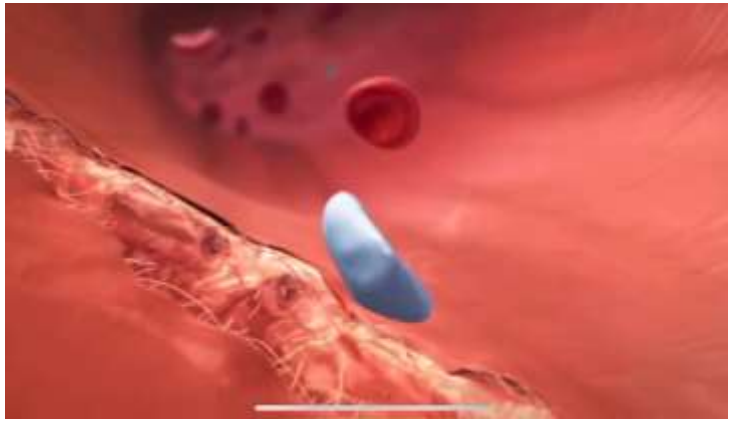
PRFM is a next generation biological product, high concentrated platelets, Growth factors and natural fibrin speeding up the healing process.

Contain active protein like fibrin, fibronectin and vitronectin

Bind within developing fibrin mesh or to the ECM

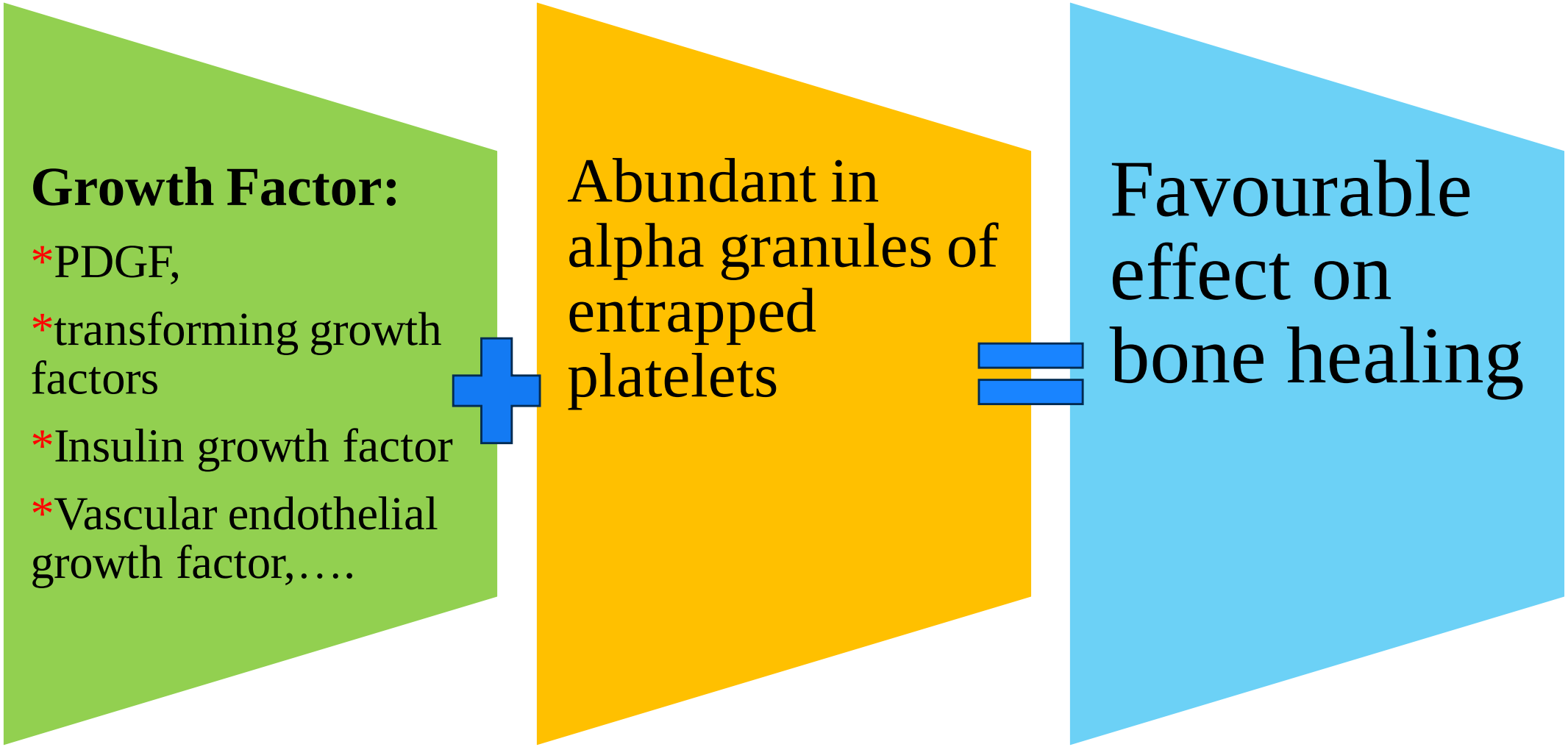
Create chemotactic gradient

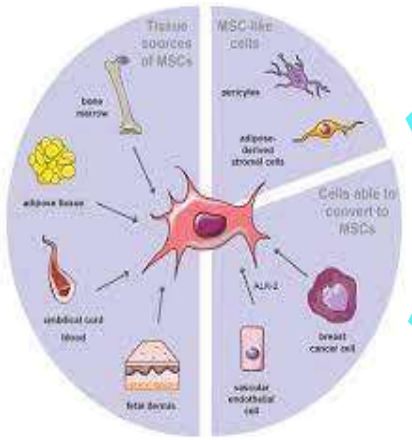
Encourages stem cells, stimulate stem cell migration ,differentiation and promoting repair



Platelets play a key role in wound healing

Platelets can play a crucial role in bone regeneration as they are reservoirs of growth factors and cytokines which are the key factors for bone healing



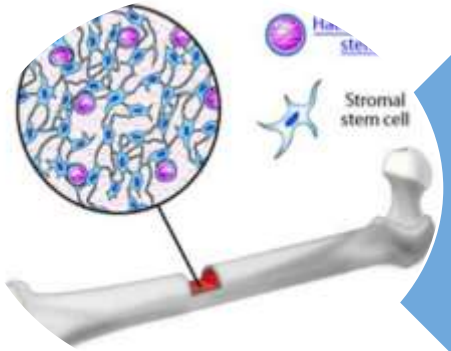


Mesenchymal stem cells (MSCs)

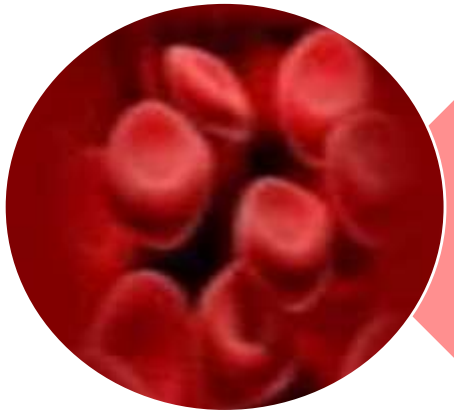
* Multipotent stromal cell with prominent regenerative functions.

* Its first identified and isolated from bone marrow and then found in various tissues including umbilical cord, adipose tissue and peripheral blood.

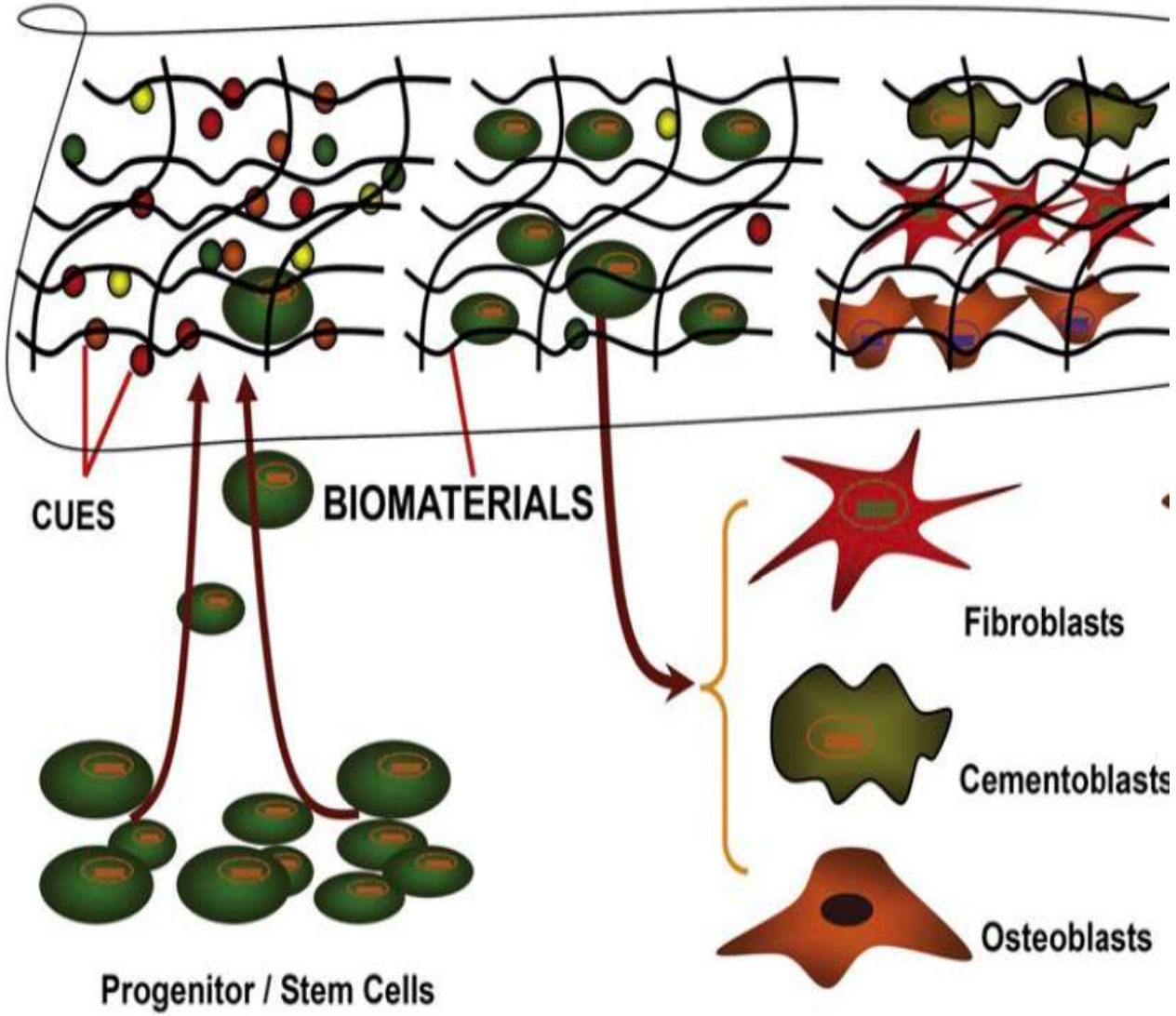
* Peripheral blood MSCs draw increasing attention as they share similar biological characteristics with MSCs derived from bone marrow or adipose tissue.



MSCs: Capable of differentiating into osteoblasts, chondrocytes, adipocytes, fibroblasts, tenocytes, and myoblasts, which are considered as a cell source for various tissue repair and regenerating bone defects.

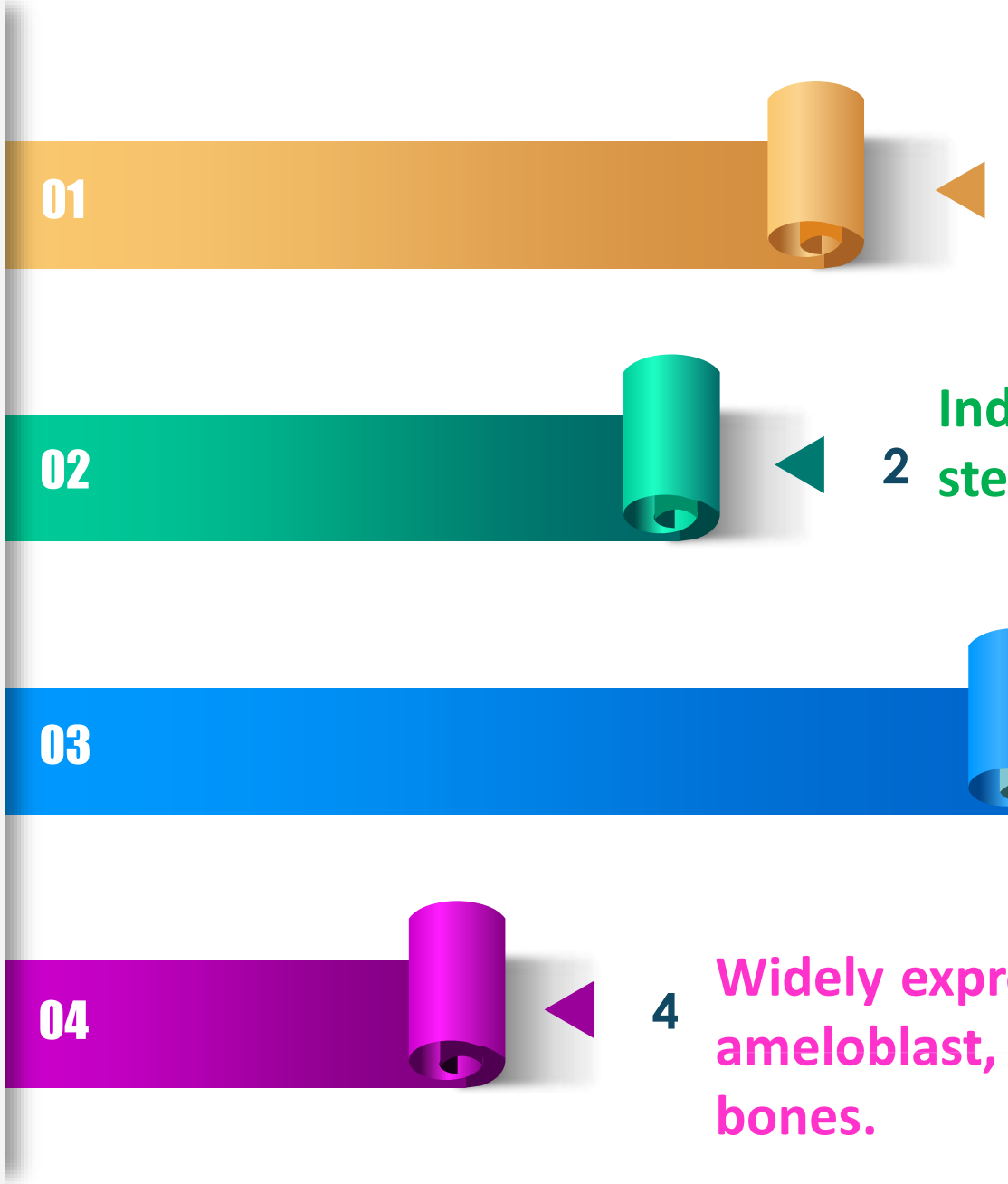


The requirements of aspiration of bone marrow from the patient will cause pain and morbidity of the donor sites. It will be very convenient if PBMSCs could be harvested and expanded to enough numbers.



The corporation in the biomaterials and stem cell within the cells reface for proliferation, differentiation, tissue growth and natural process of bone healing.

BMP9



1 Belongs to the transforming growth factor beta superfamily.

2 Induces the differentiation of mesenchymal stem cells (MSCs) to an osteoblast lineage.

3 Play an important role on tooth development

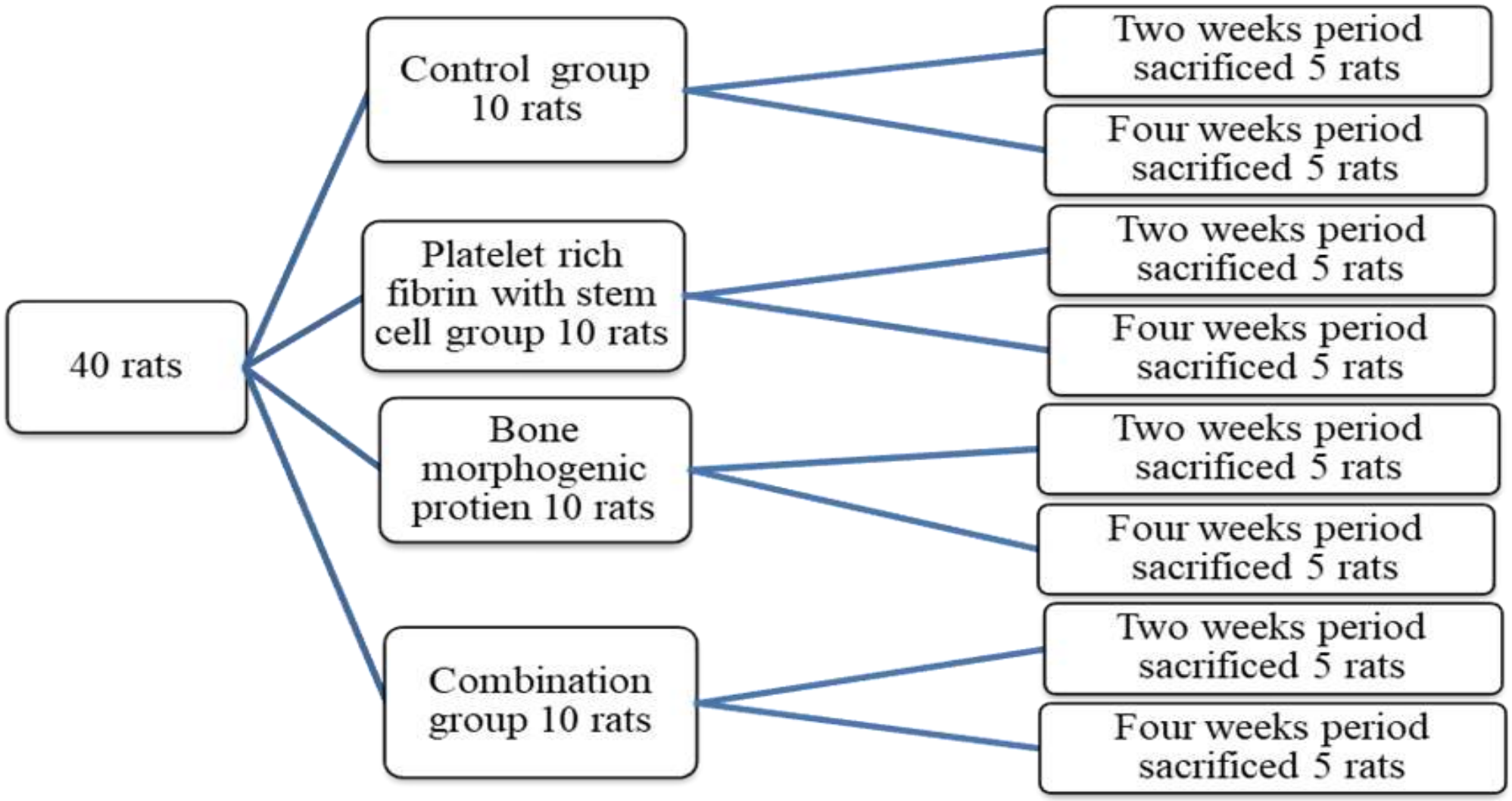
4 Widely expressed in osteoblast, odontoblast, ameloblast, dental pulp cells and alveolar bones.

Flow cytometry: Used to evaluate cells for a number of functions, such as cell counting, sorting, phenotyping…..

CD34 and CD44 are protein markers that are present on the surface of stem cells and differentiates peripheral blood mesenchymal stem cell.

MATERIALS AND METHODS

Study design



Peripheral blood mesenchymal stem cells

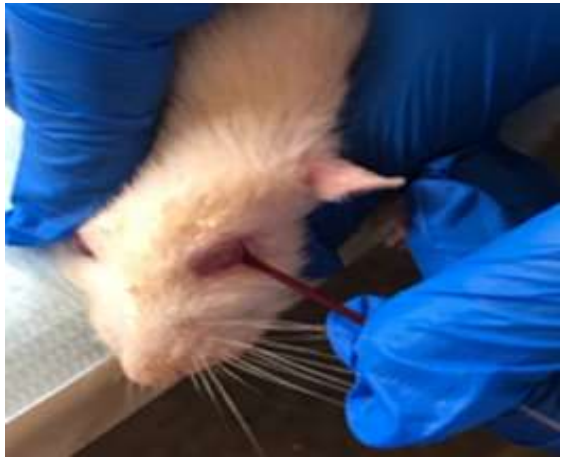
Platelet rich fibrin matrix

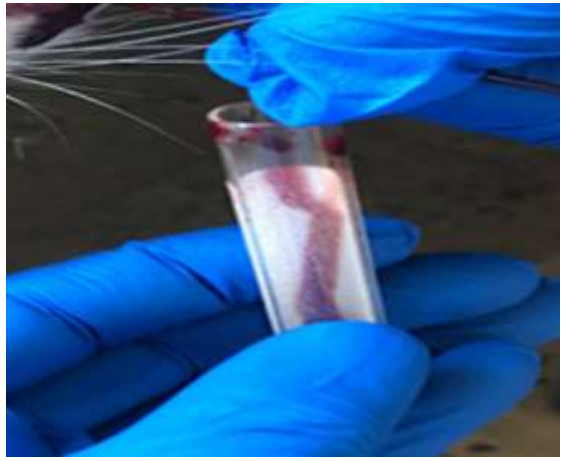
Supercell peripheral blood mesenchymal stem cells and platelet rich fibrin matrix

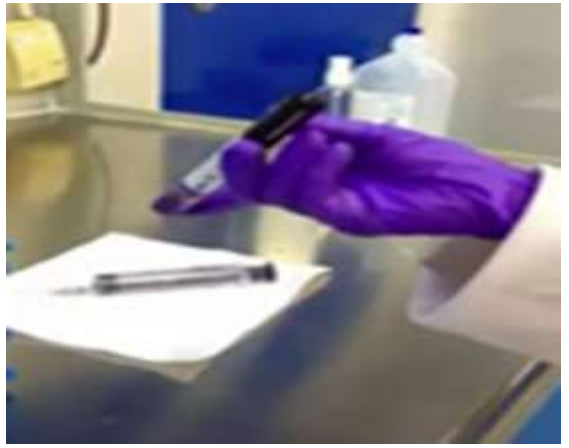
Preparation of Stem cells

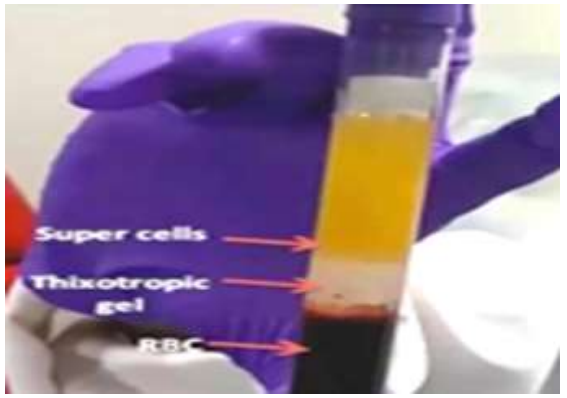
1. A 3ml blood sample was obtained from the orbital sinus of each rat of the corresponding group. Using collection tube containing a cell separator gel.
2. Mix the blood 2-3 time. The tube is then place into centrifuge and spun for 5 minutes at 3000 rpm
3. Separate the blood into supernatant plasma/stem cells suspension. The red blood cell are located below the cell separator gel.

The stem cells i.e.(0.2ml -0.3ml) just a above the gel is kept aside for further use.

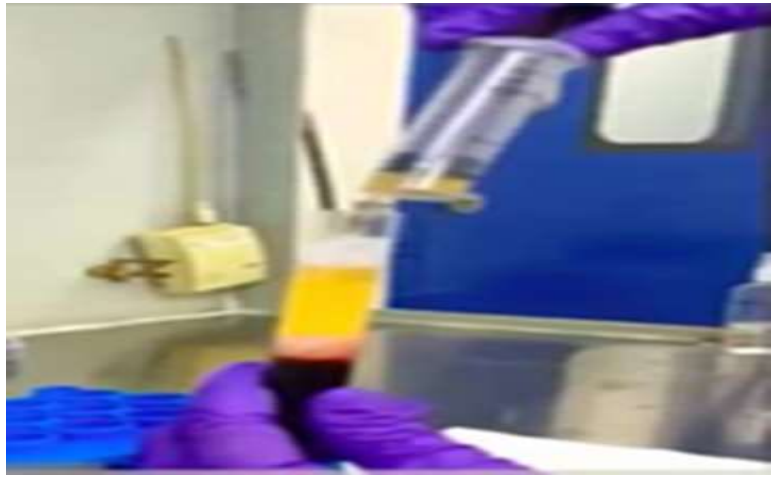


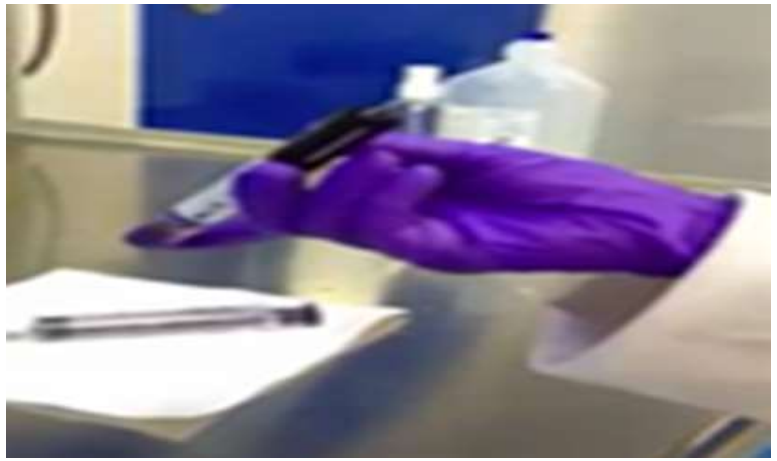






4. Discard of upper layer of plasma and collected the lower 0.2ml-0.3ml of the stem cell just above the gel
5. Invert the tube in order to mix the cells present on the surface of the gel with the plasma
6. Carefully collect 0.2ml-0.3ml of plasma containing the super cell and aspirate into another tube and keep it aside for further used



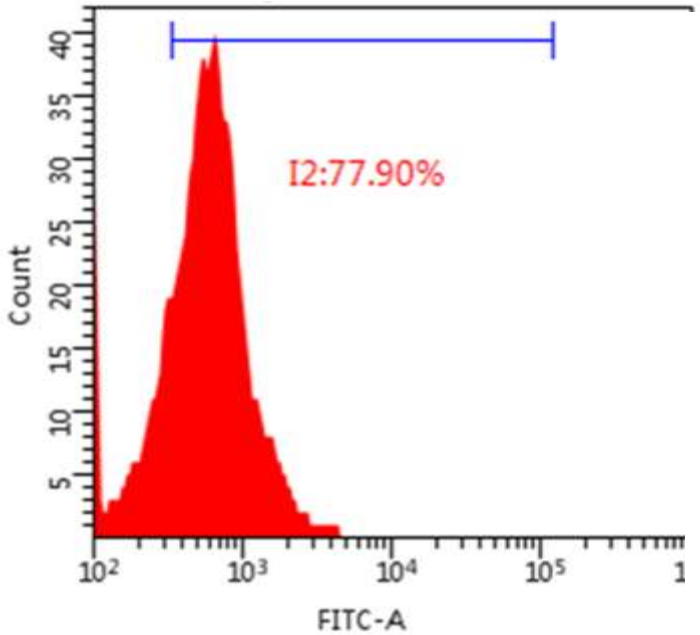


Preparation of PRFM glue

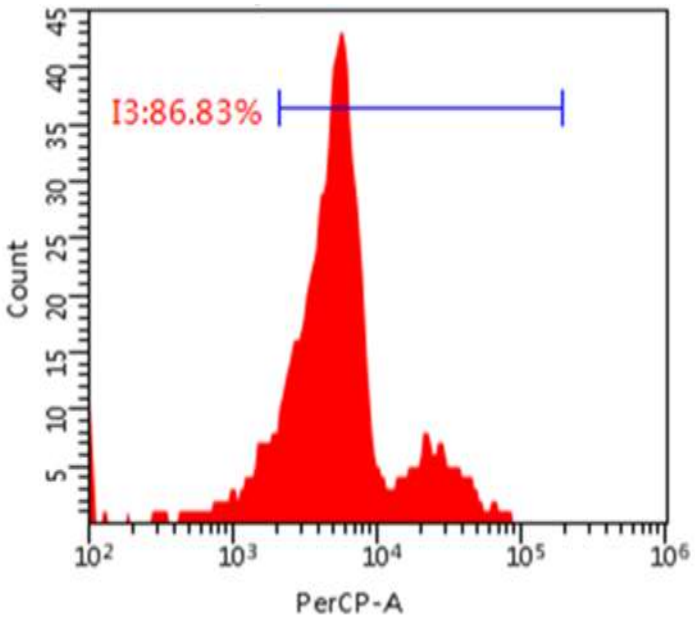
1. A small amount of blood (1ml whole blood) is drawn into collection tube containing a gel separator.
2. The stem cells prepared previously is mixed to the whole blood in the gel separator tube.

*At this point of time identify the stem cells present by using flow cytometric analysis and check CD 34,CD 44 as CD marker.

Positive expression of MSCs markers.

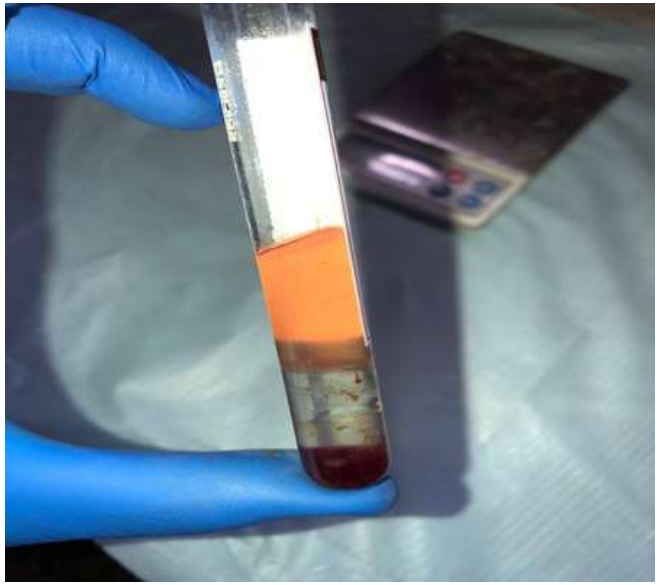


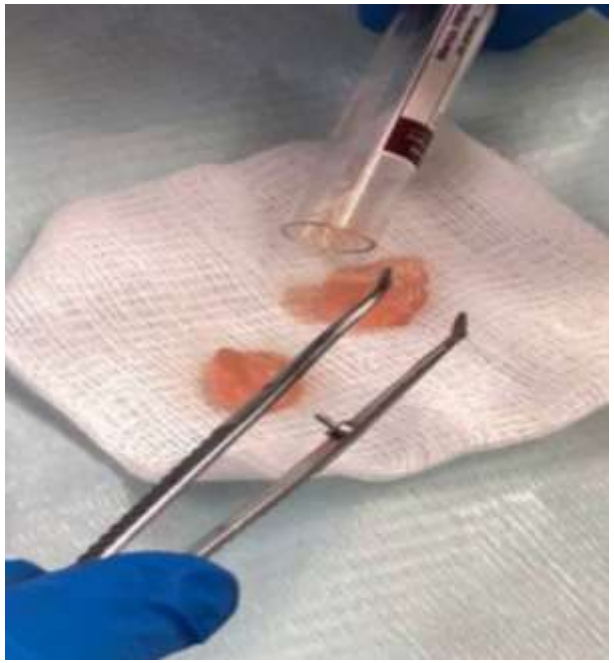
CD34



CD44

3.The tube is then placed into a centrifuge and spun for 6 minutes 3400 rpm to separate the blood into a supernatant plasma / platelet suspension.
4.The red blood cells are located below the gel.
5.After centrifuge due to fibrin polymerization PRF is formed.
6.The PRF clot was immediately withdrawn from the tube using sterilized tweezer .
PRF clot was then placed between two layers of sterile gauze with gentle pressure applied to squeeze fluids out thus finally obtaining PRF membrane

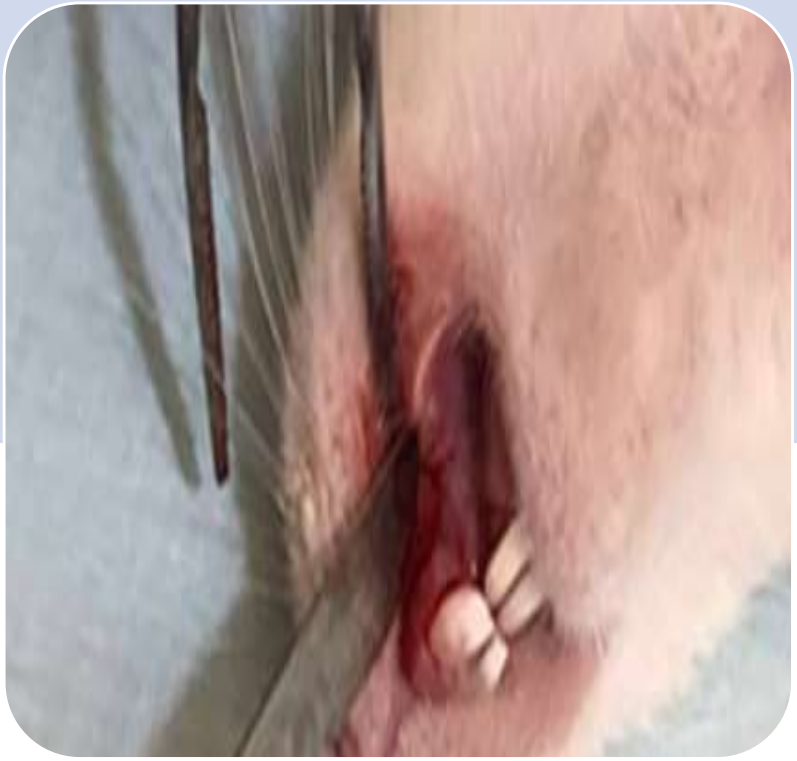




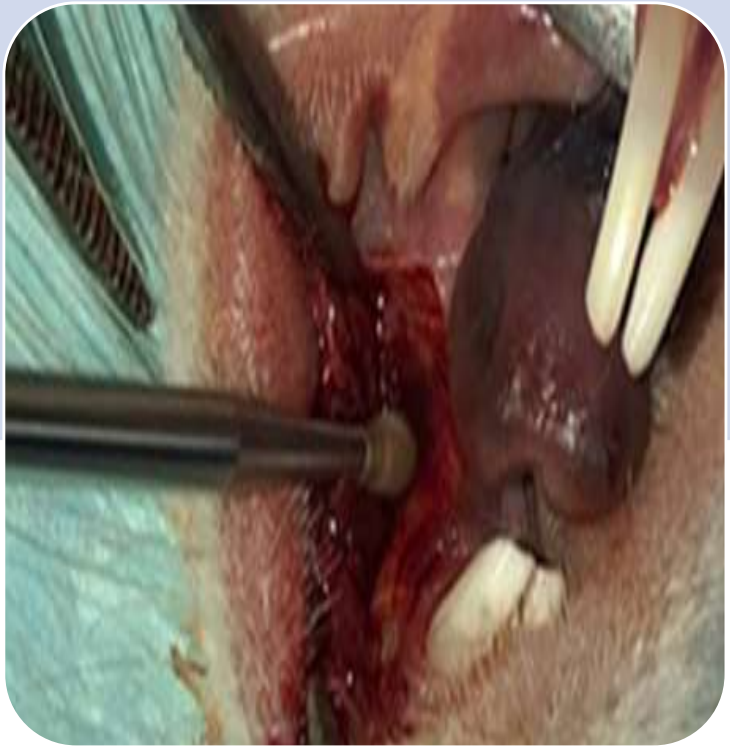
BMP9 active protein preparation:

- Added (100 microliter) phosphate buffer saline to 0.1mg (active protein).
- A quick spin of the vial (vortex) followed by reconstitution in phosphate buffer saline to a concentration not less than (1 mg/ml).
- This solution can be diluted into other buffers.

Surgical procedure and intervention

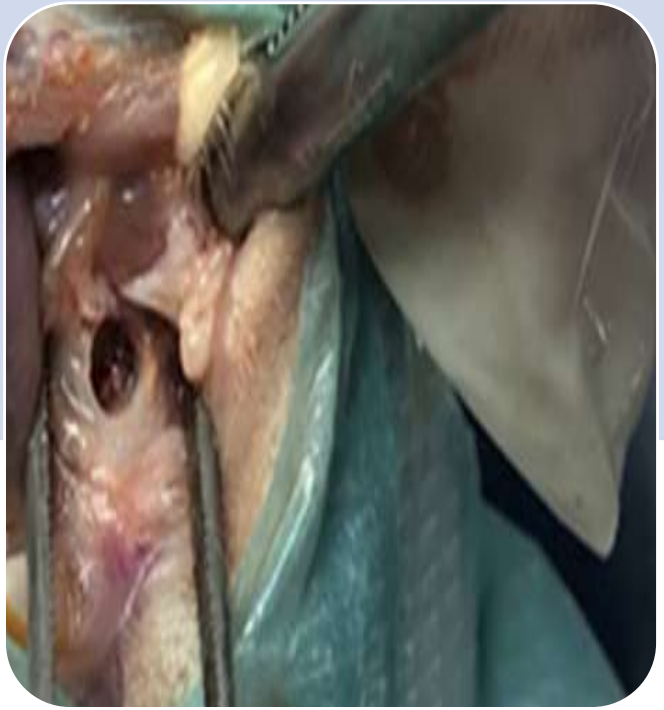


Incision

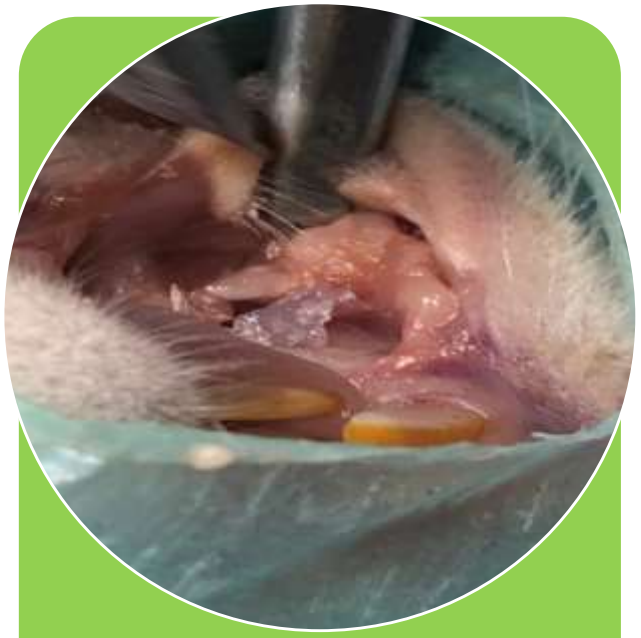


Penetration

(dimention 2mm, depth 3mm)

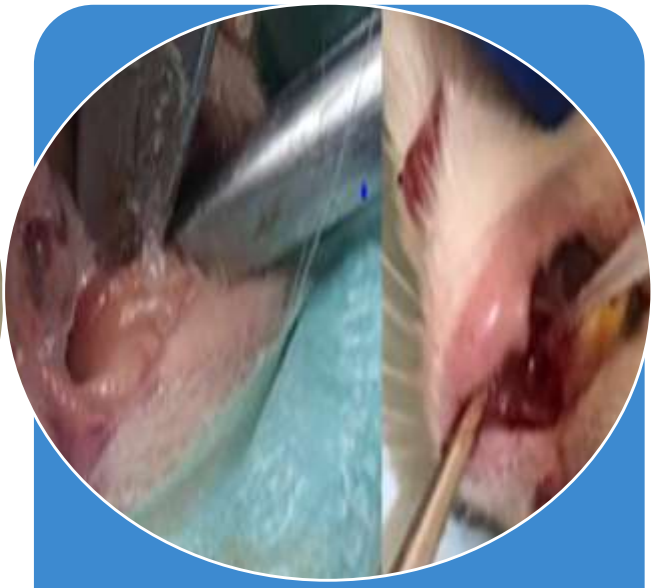


Intra-bony hole

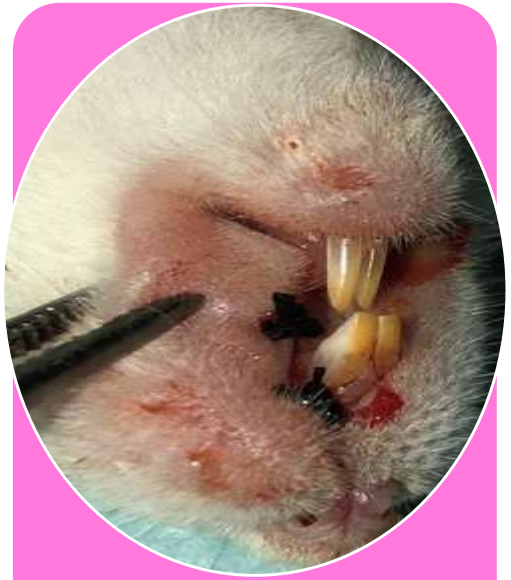


Group A treated with PRFM with stem cell

Group B treated with active protien (BMP9)



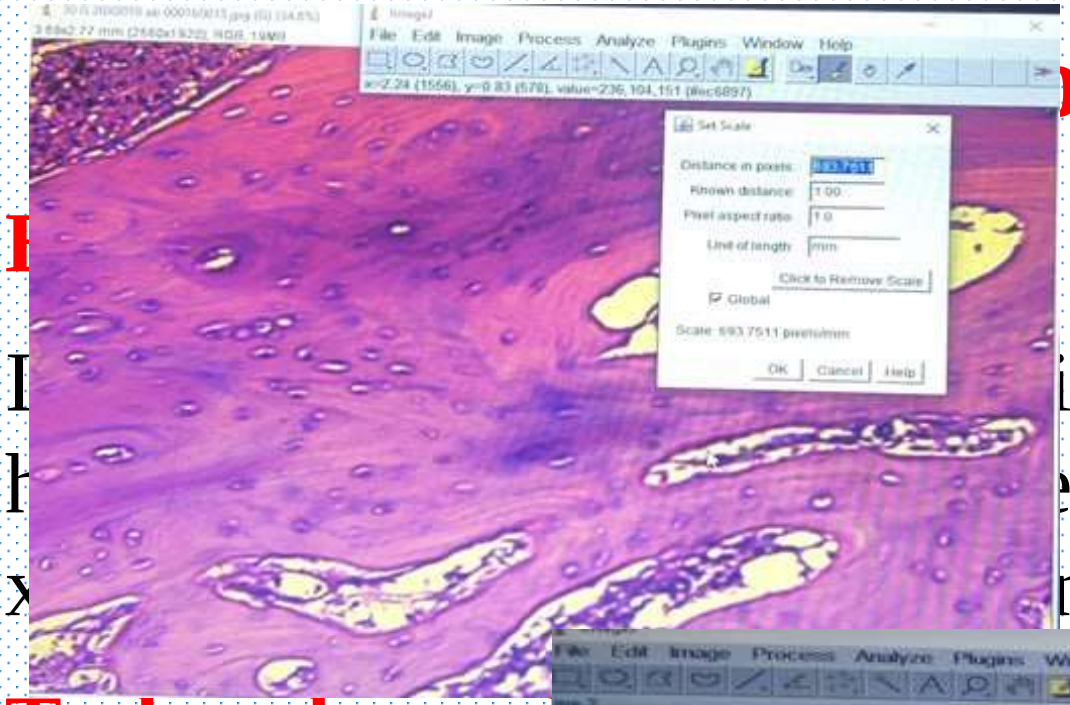
Group AB treated with PRFM with stem cell and BMP9

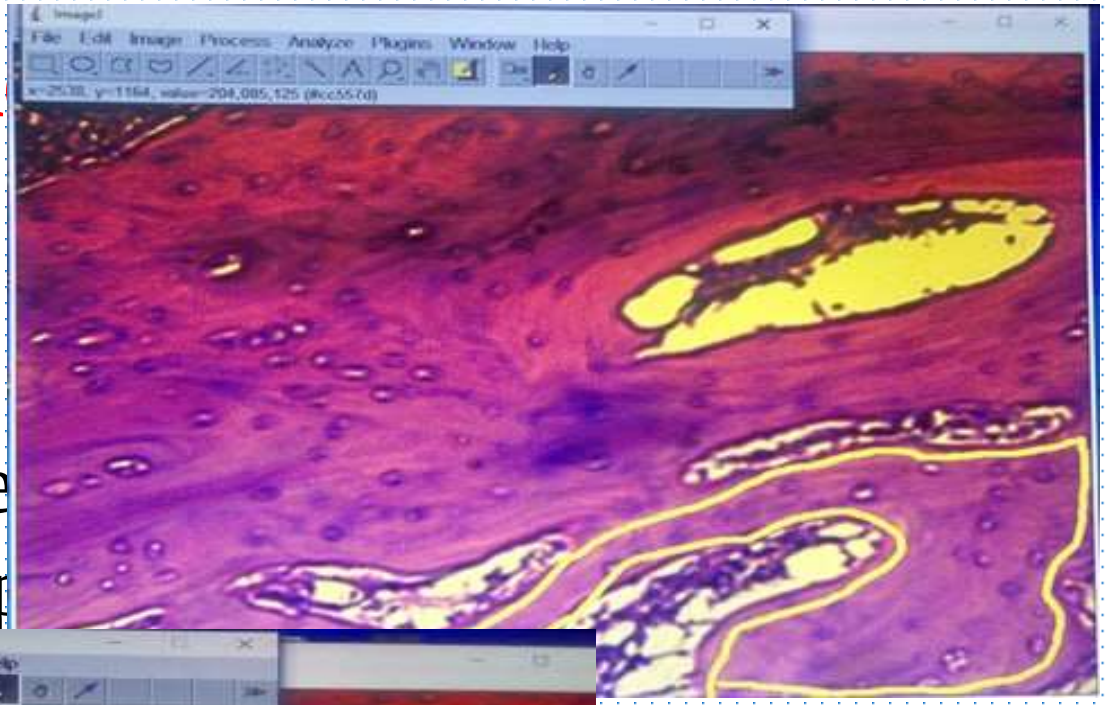


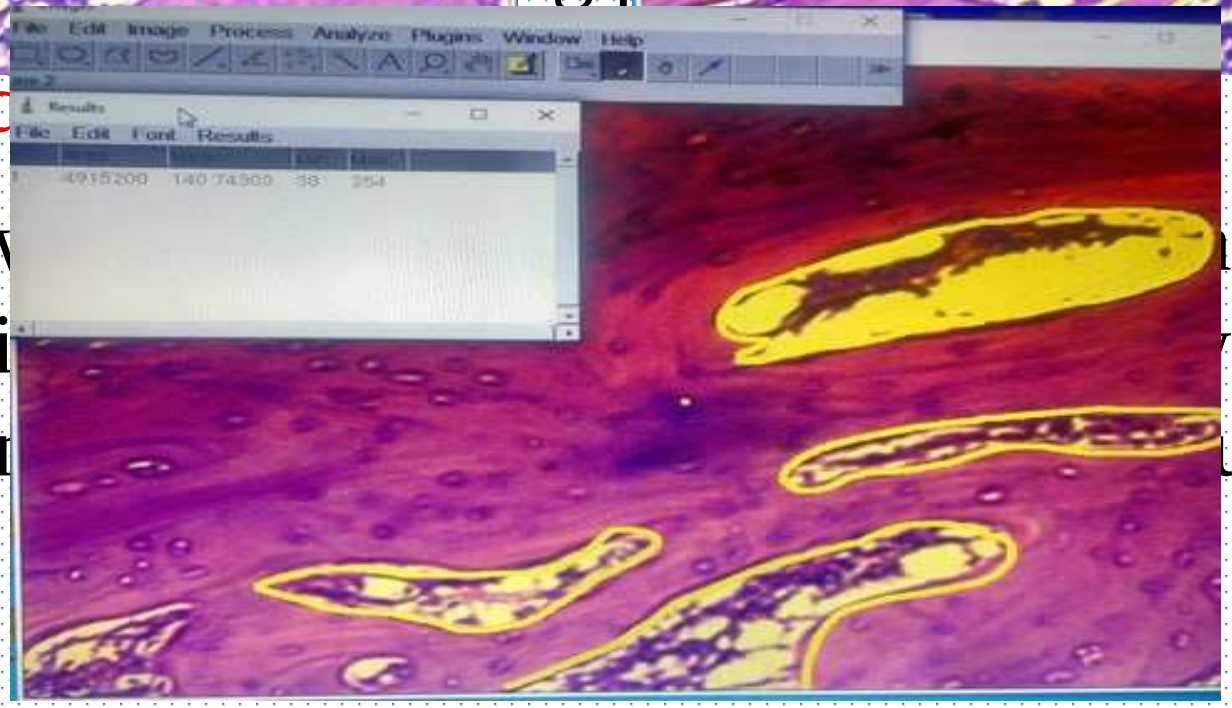
Suturing

HISTOLOGICAL PREPARATION

1.Fixation	10% neutral formalin
2.Decalcification	10% formic acid
3.Dehydration	Alcohol
4.Embedding	Wax
5- Sectioning	4 µm section for H&E
6-Staining	H&E
7-Examination of the slides under light microscope	







om

Trabecular and

Measurements v m imageJ.exe

Two microscopi ere taken by a

microscopic ca upper parts to

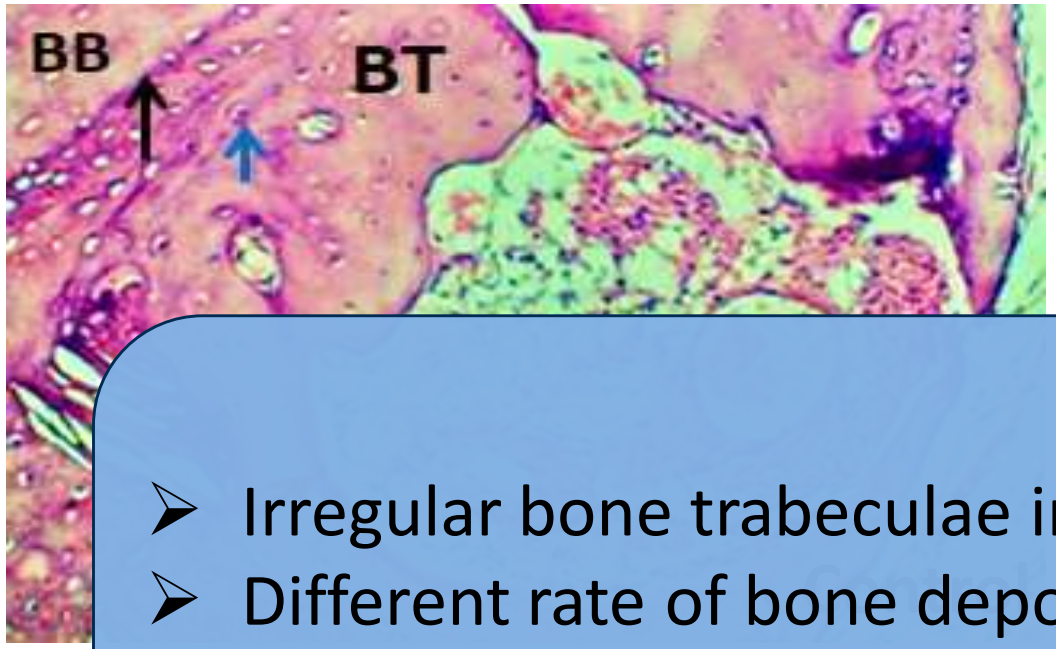
cover all defect

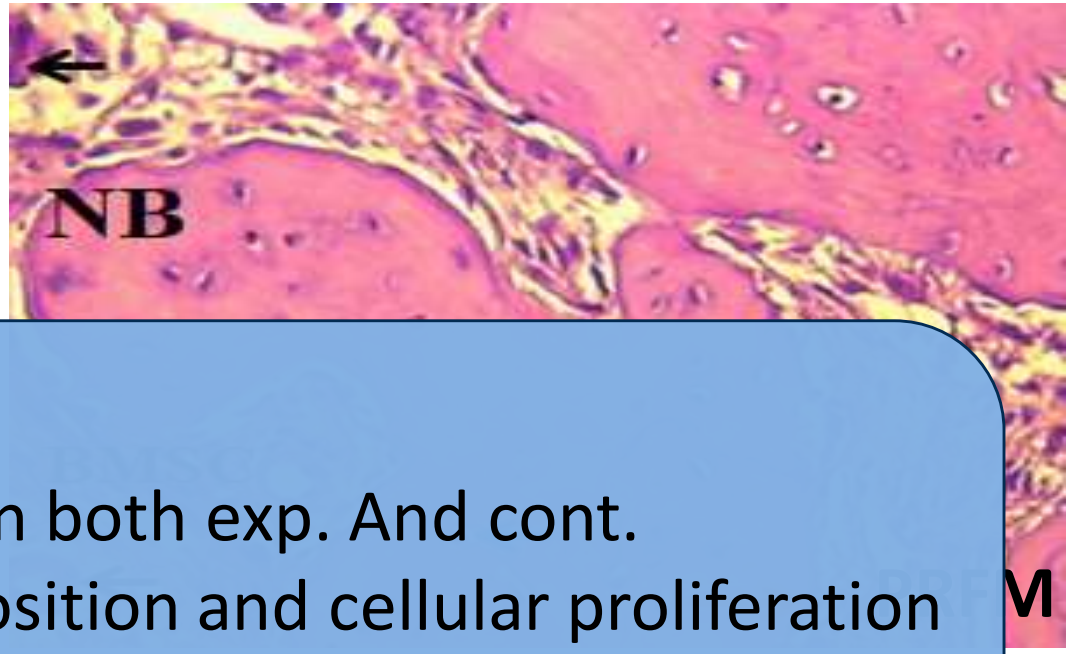

RESULT

&

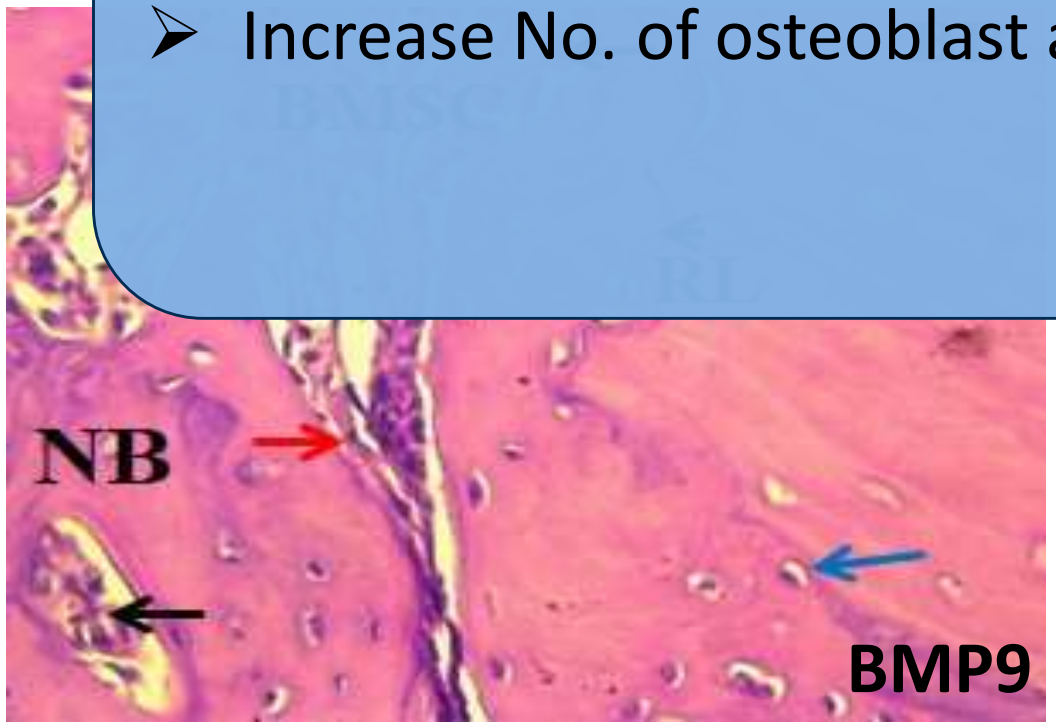

DISSCUSION

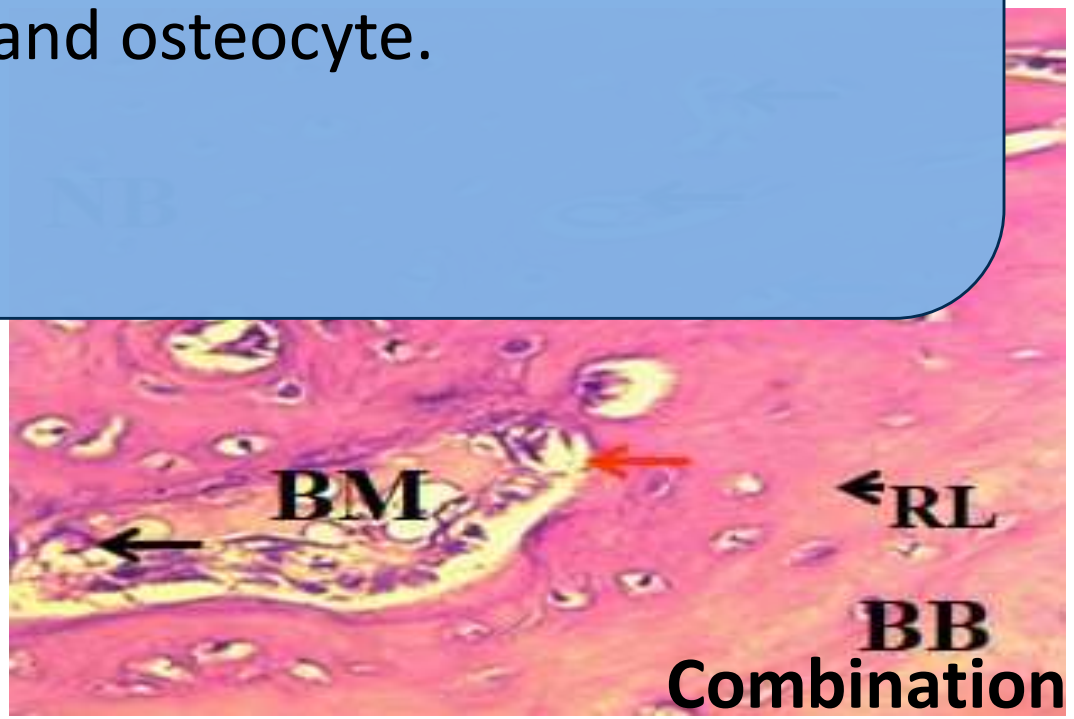
Histological findings (H & E stain): At 2 weeks duration:





- Irregular bone trabeculae in both exp. And cont.
- Different rate of bone deposition and cellular proliferation
- Increase No. of osteoblast and osteocyte.





Agree with:

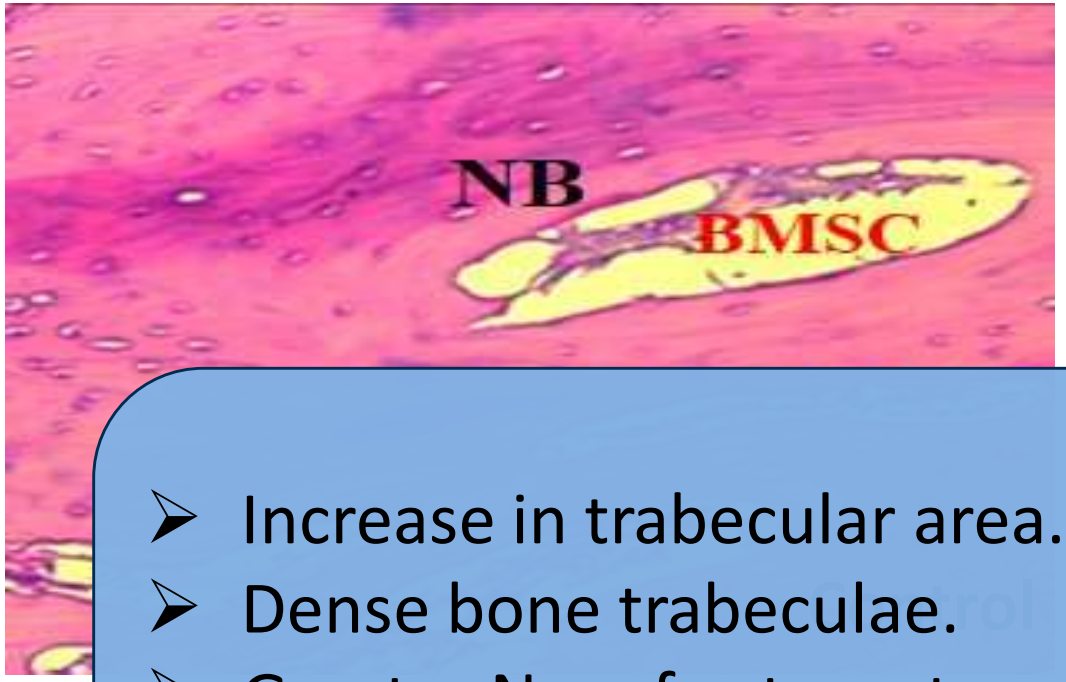
- Salih et al., 2018
- Lee et al., 2022
- Zhon et al 2020
- Xiao et al., 2020

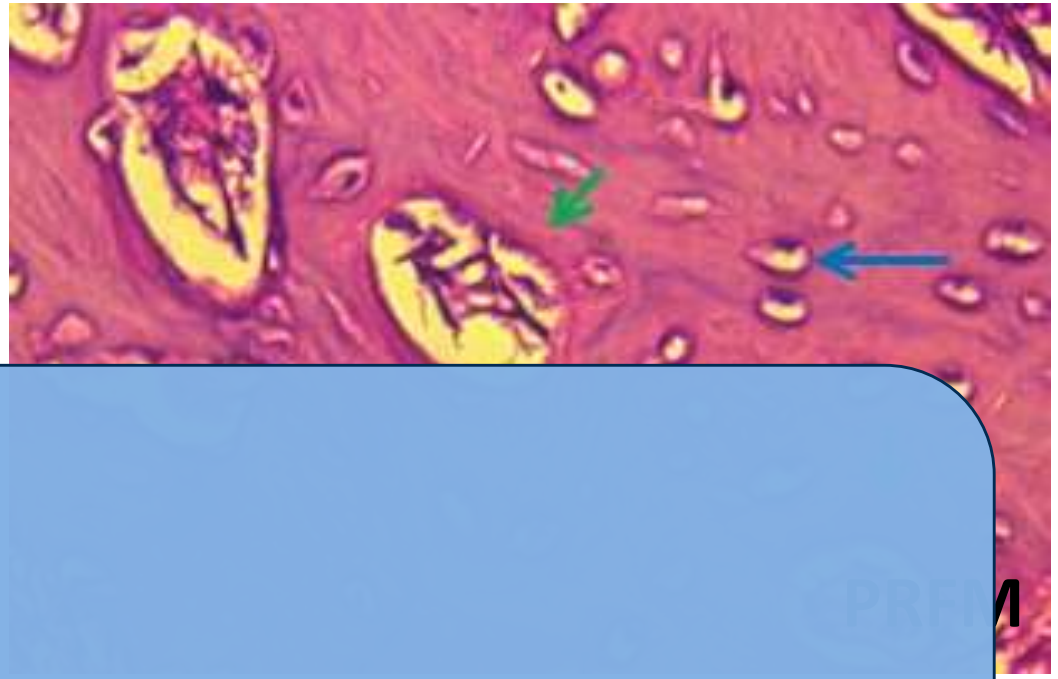
Disagree with:

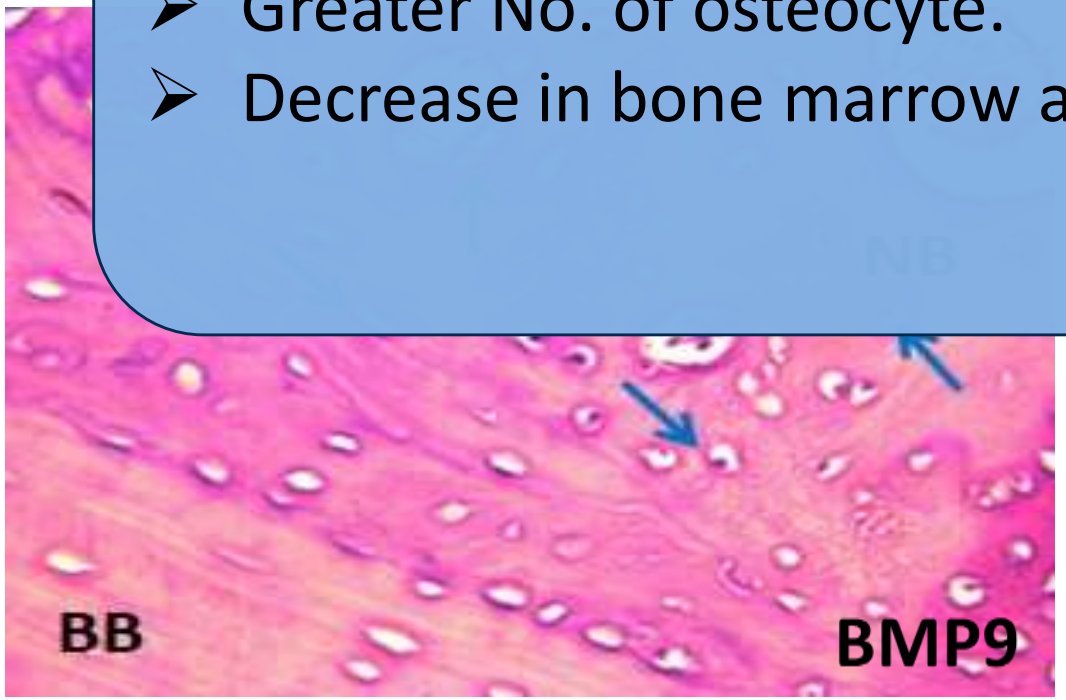
- Fong et al., 2013

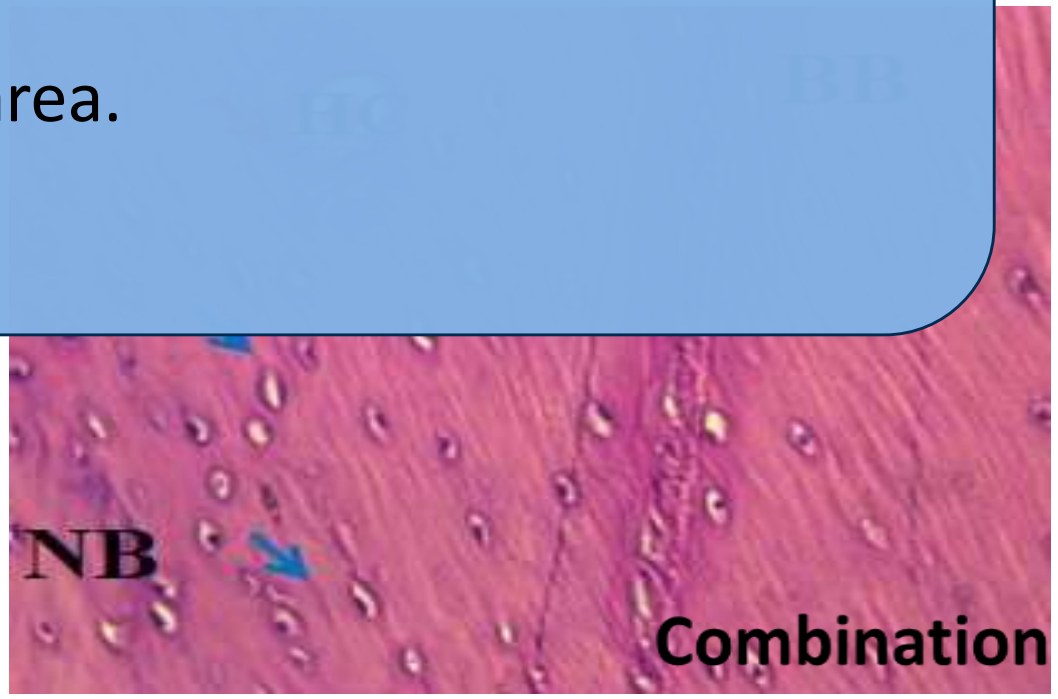
Histological findings (H & E stain): At 4 weeks duration:

- Increase in trabecular area.
- Dense bone trabeculae.
- Greater No. of osteocyte.
- Decrease in bone marrow area.









Agree with:

- Fadhil et al., 2023
- Lee et al., 2023
- Nour & Aggour, 2018

Osteocyte

Duration	Groups	Descriptive Statistics			
		Mean	S.D.	Min.	Max.
2 weeks	Cont.	24.1167	1.83893	21.80	26.70
	A	24.6000	2.67507	21.80	29.40
	B	23.3667	2.44431	20.90	27.40
	AB	31.9667	4.16781	23.80	35.20
4 weeks	Cont.	34.6333	1.54747	32.30	36.20
	A	38.6333	2.28532	35.50	41.40
	B	42.8500	.52440	42.20	43.80
	AB	46.7667	1.53058	45.10	49.40

Osteoblast

Duration	Groups	
		Mean
2 weeks	Cont.	37.2000
	A	41.8500
	B	41.5833
	AB	48.0333
4 weeks	Cont.	29.8333
	A	26.7000
	B	25.5667
	AB	19.6333

Oseoclast

Duration	Groups	Descriptive Statistics				Comparison	
		Mean	S.D.	Min.	Max.	F-test	P- value
2 weeks	Cont.	1.5500	.36194	1.00	2.00	6.786	.002**
	A	1.1833	.61779	.50	2.00		
	B	.7500	.28810	.30	1.10		
	AB	.5167	.38687	.00	1.00		
4 weeks	Cont.	.5500	.18708	.30	.80	5.748	.005**
	A	.4667	.18619	.20	.70		
	B	.2500	.18708	.00	.50		
	AB	.1833	.14720	.00	.40		

Agree with

- Wang et al., 2022
- Dvorakova et al., 2023
- Freeitas et al., 2021

CONCLUSION

AND

Conclusion

1

Histological evaluation exhibit the combination of PRFM with stem cells and bone morphogenic protein 9 (BMP9) had positive effect on bone healing by increasing proliferation of osteoblast cells lead to increase bone deposition and mineralization.

2

The present study showed that the PRFM with stem cells/BMP9 are officinal and their combination were effective in the promotion of osteogenesis which may encourage future clinical surgical utilization

1

2

Suggestions

1

Investigate the localization of other bone formation marker such as bone specific alkaline phosphatase, or osteocalcin in bone healing treated with combination of PRFM with stem cells and BMP9.

2

As recent research can improved the PRFM with stem cells, BMP9 expression by gene analysis by real time PCR.

3

Evaluate the effect of combination of PRFM with stem cells and BMP9 on osseointegration around dental implants.


Thank you