Engineering Bone Tissue from Human Embryonic Stem Cells

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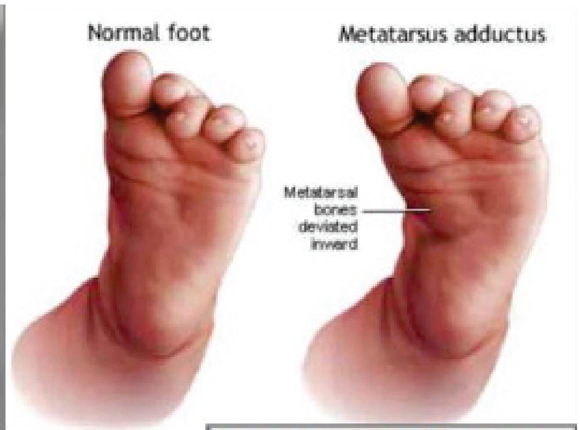
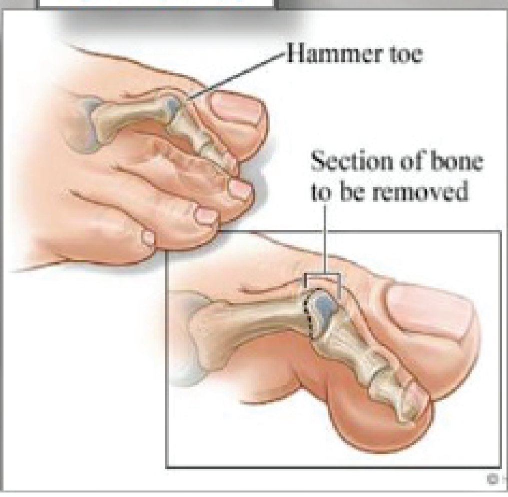
Bone defects from Congenital and trauma malformations.



Metatarsus adductus

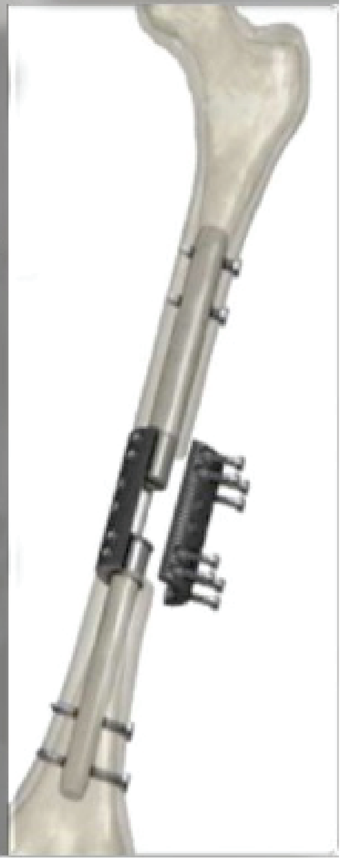
Hammer Toe



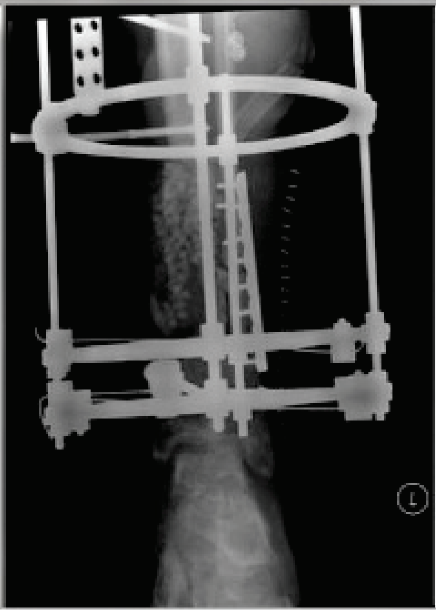
Bunion's feet

Current strategies in treating bone defects

Osteobridge: Stabilization of bone defects



Illizarov frame: To treat bone deformations or fractures.



Problems with conventional methods

- **Extremely Traumatic .**
- **Not so economical use of mechanical and structurally competent scaffolds.**
- **Requirement of synergistic development of vascular supply and bone to maintain viability.**

Alternative

Use of Human Embryonic Stem cells in engineering bone tissue custom made for individuals thus easing the process in avoiding graft rejection related issues.

Safe to use and time saving by generating the bone tissue on an increased time scale.

Hypothesis

Cultivation of hESC-derived Mesenchymal Progenitor Cells (MPCs) on 3D Osteoconductive scaffolds in perfusion reactor system leads to formation of large and compact bone constructs.

Bone Tissue engineering protocol and timeline

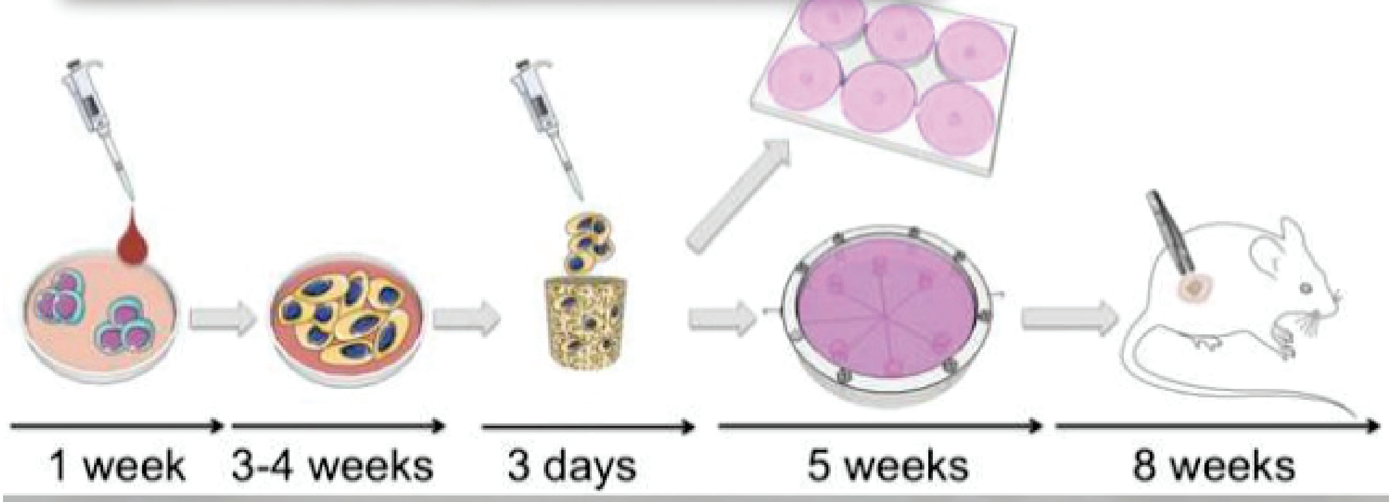
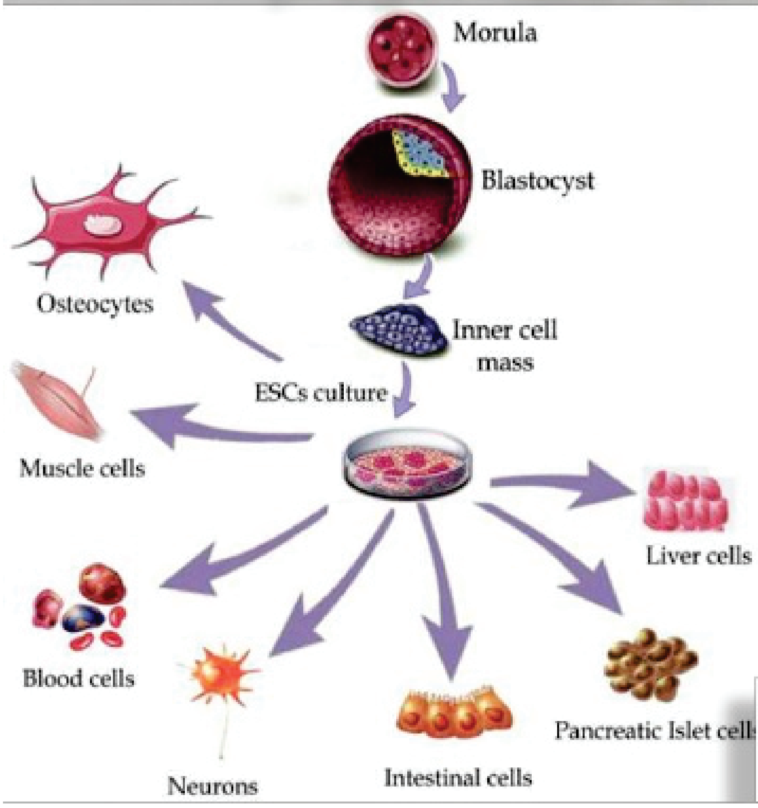


Fig 1: Bone Engineering protocol and time line.

Specific aims

- Aim 1: Derivation of Mesenchymal progenitors from hESCs.
- Aim 2: Culturing MPCs on 3D scaffolds in perfusion bioreactor system.
- Aim 3: Effects of Reactor cultivation on MPCs in tissue development.
- Aim 4: *In vivo* Safety and stability analysis of the engineered bone construct.

Aim 1: Derivation of Mesenchymal progenitors from hESCs.



- **Differentiation is induced in two hESC Cell lines – H9 and H13.**
- **Mesenchymal Differentiation potential was analysed and suitable cell line was selected for further analysis.**

Differentiation of hESCs into different cell types.

- H9 and H13 cultures were found to show continuous growth.
- Mesenchymal surface antigens (Blue box) were expressed on progenitors.
- Further analysis on the cell lines were carried out.

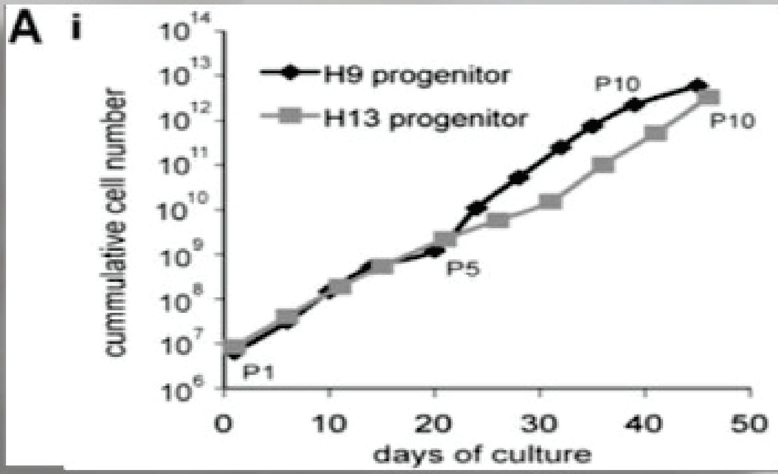


Fig S1C: Surface antigen expression of hESC progenitors

Surface antigen	H9-progenitor					H13-progenitor				
	P1	P3	P5	P7	P10	P1	P3	P5	P7	P10
SSEA-1	-	-	-	-	-	-	-	-	-	-
SSEA-4	-	-	-	-	-	-	-	-	-	-
CD31	-	-	-	-	-	-	-	+/-	-	-
CD34	-	-	-	-	-	-	-	-	-	-
CD44	+	+	+	+	+	+	+	+	+	+
CD73	+	+	+	+	+	+/-	+	+	+	+
CD90	+	+	+	+	+	+	+	+	+	+
CD105	-	+/-	-	+/-	-	-	-	+/-	+	+
CD166	+	+	+	+	+	+/-	+	+	+	+
CD271	-	-	-	-	-	-	-	-	-	-

Fig : Mesenchymal differentiation potential screened in monolayer cultures.

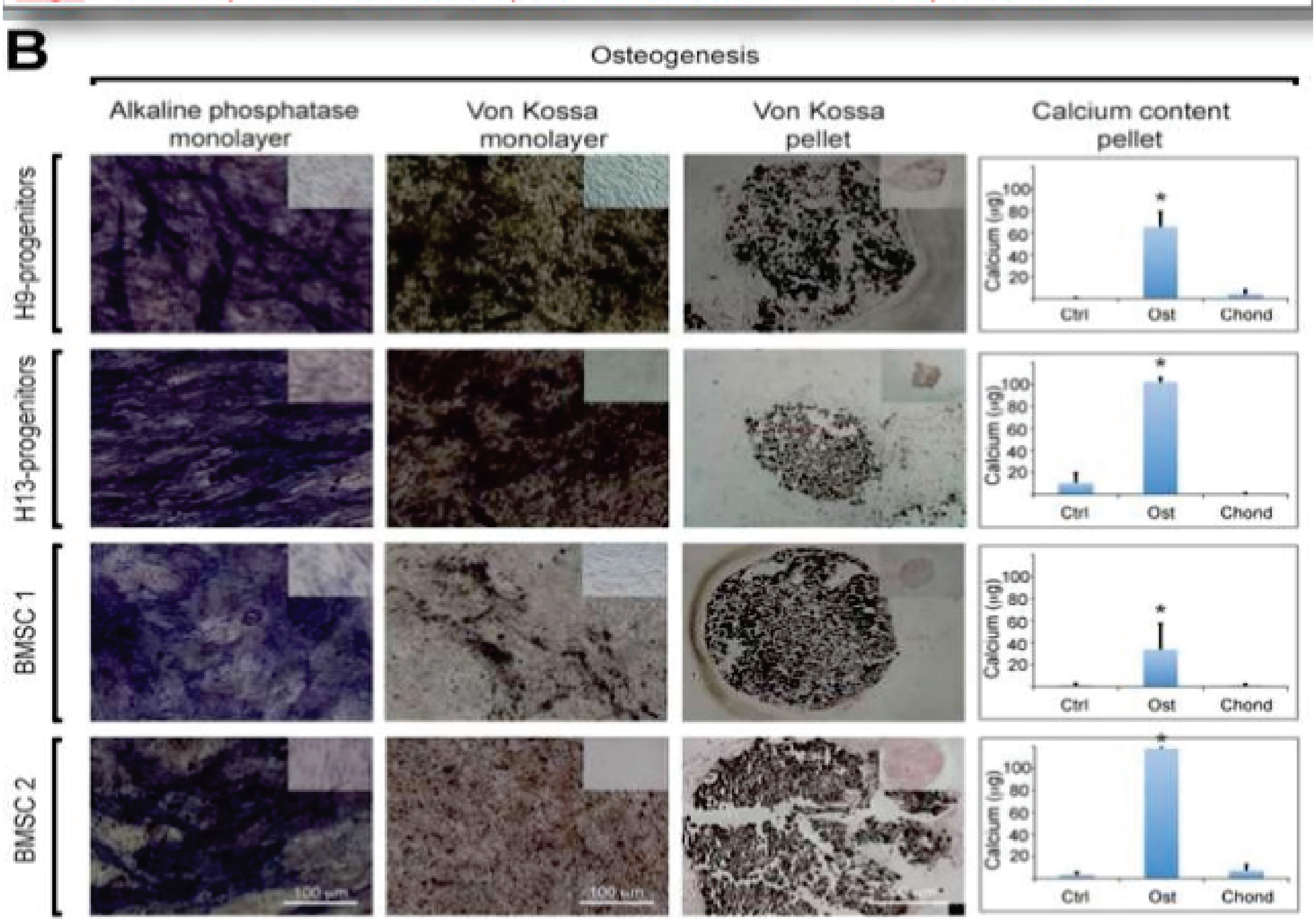
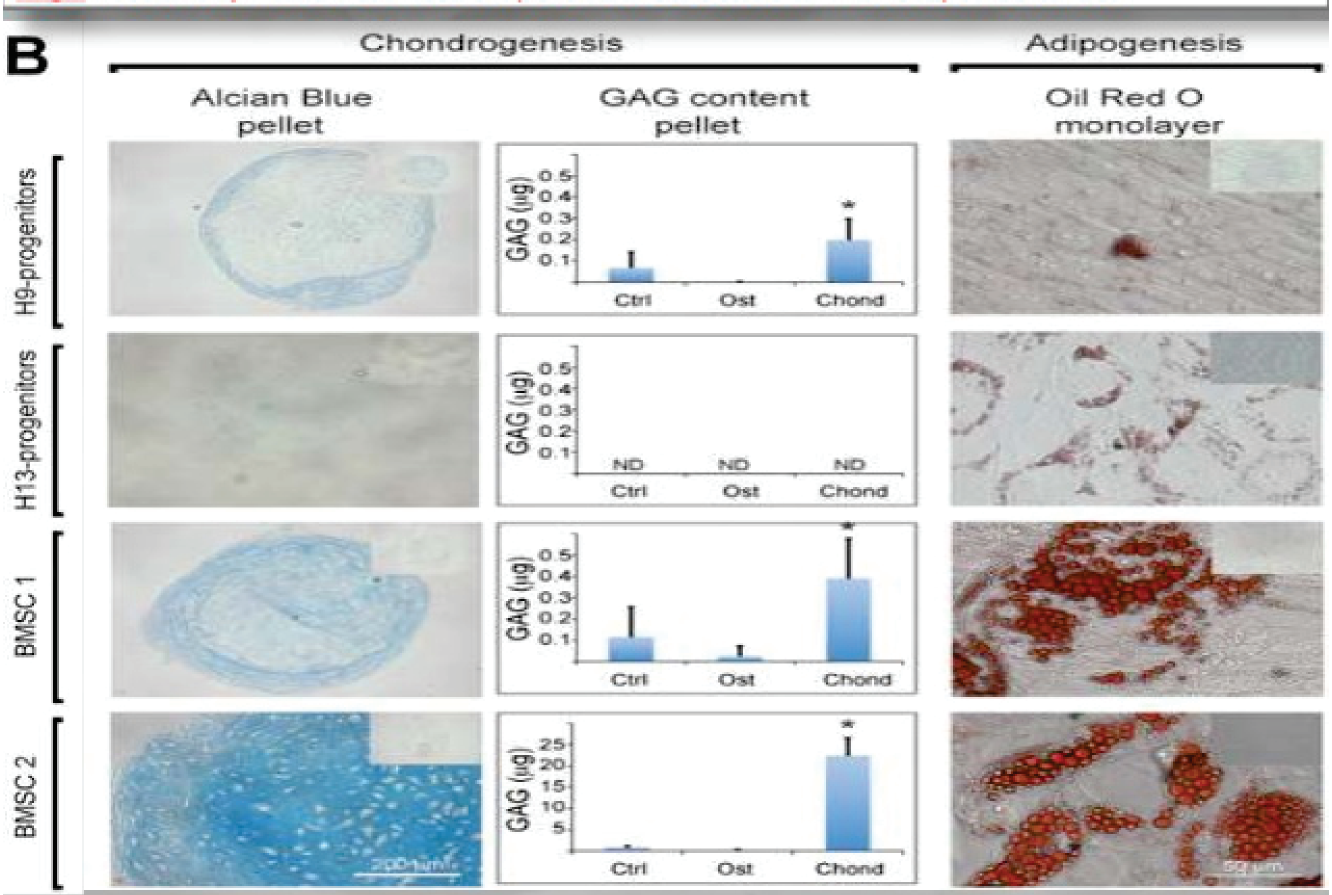


Fig : Mesenchymal differentiation potential screened in monolayer cultures.



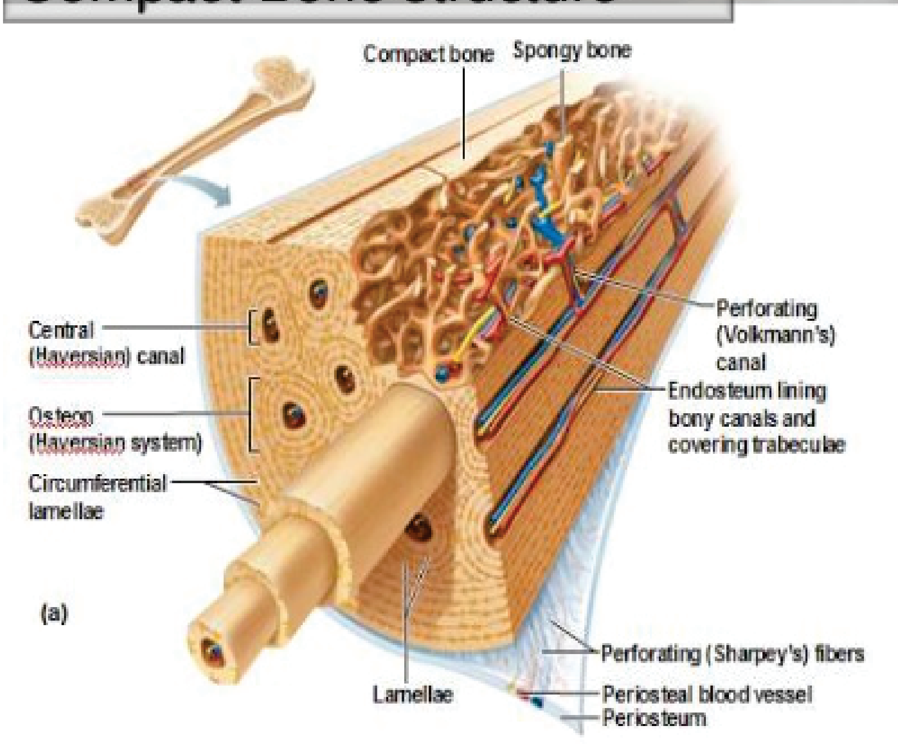
- From the tests performed H9 cultures showed
  - Lesser or zero adipogenic potential
  - Higher chondrogenic potential
  - Higher activity of Alkaline phospatase and
  - Increased matrix mineralization

  In comparison with H13 cultures and Bone Marrow derived mesenchymal stem cells (BMSCs) as positive control.

  Based on which, **H9 cultures** are selected for the long run.

Aim 2: Culturing MPCs on 3D scaffolds in perfusion bioreactor system.

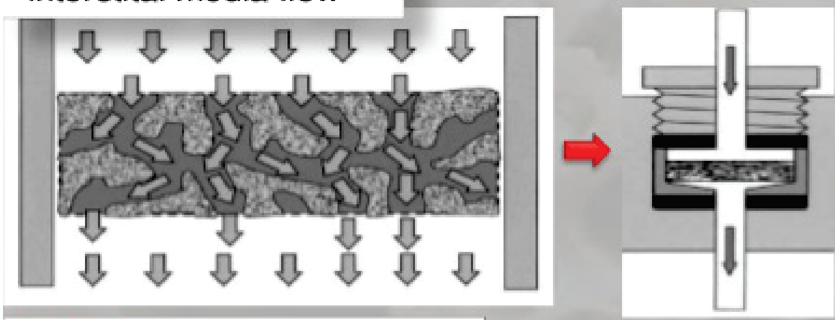
Compact Bone structure



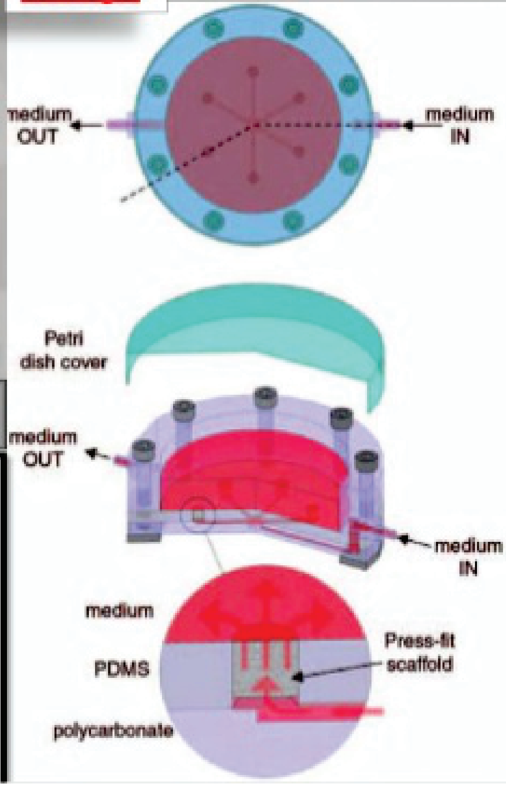
3D Scaffold Structure

Perfusion Bioreactor system

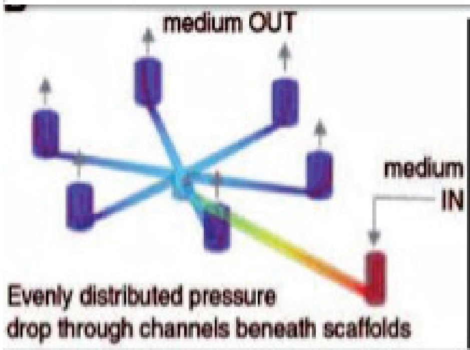
Criteria:
Interstital media flow



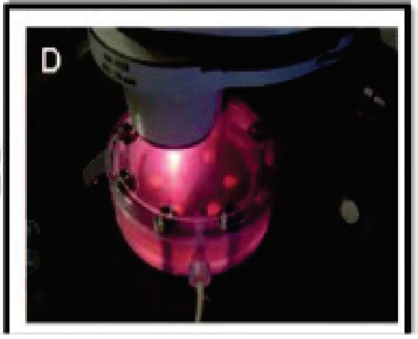
Design:



Concept:
Channeling media into scaffolds



Final End product



Aim 3: Effects of Reactor cultivation on MPCs in tissue development.

Fig - 2

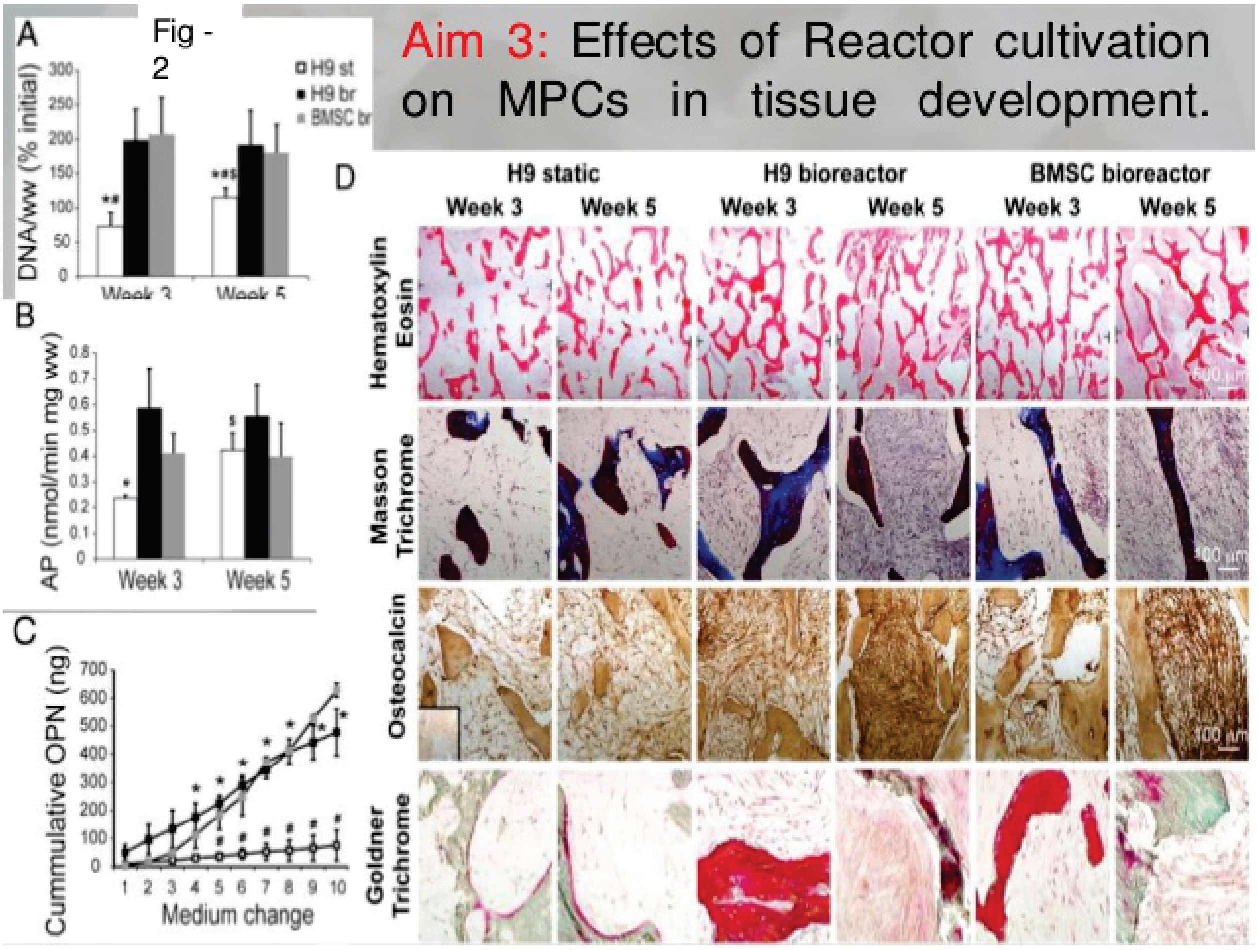
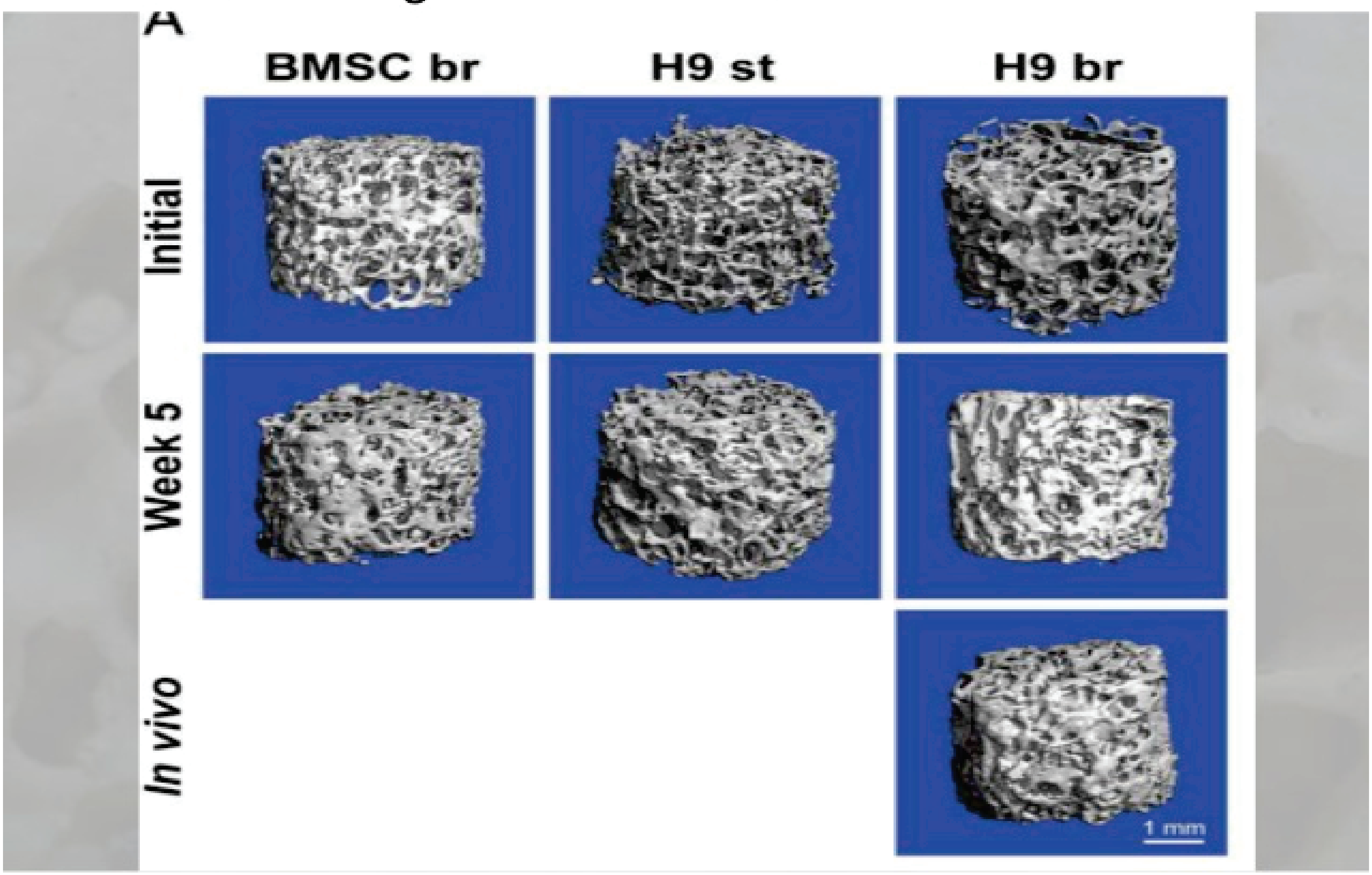
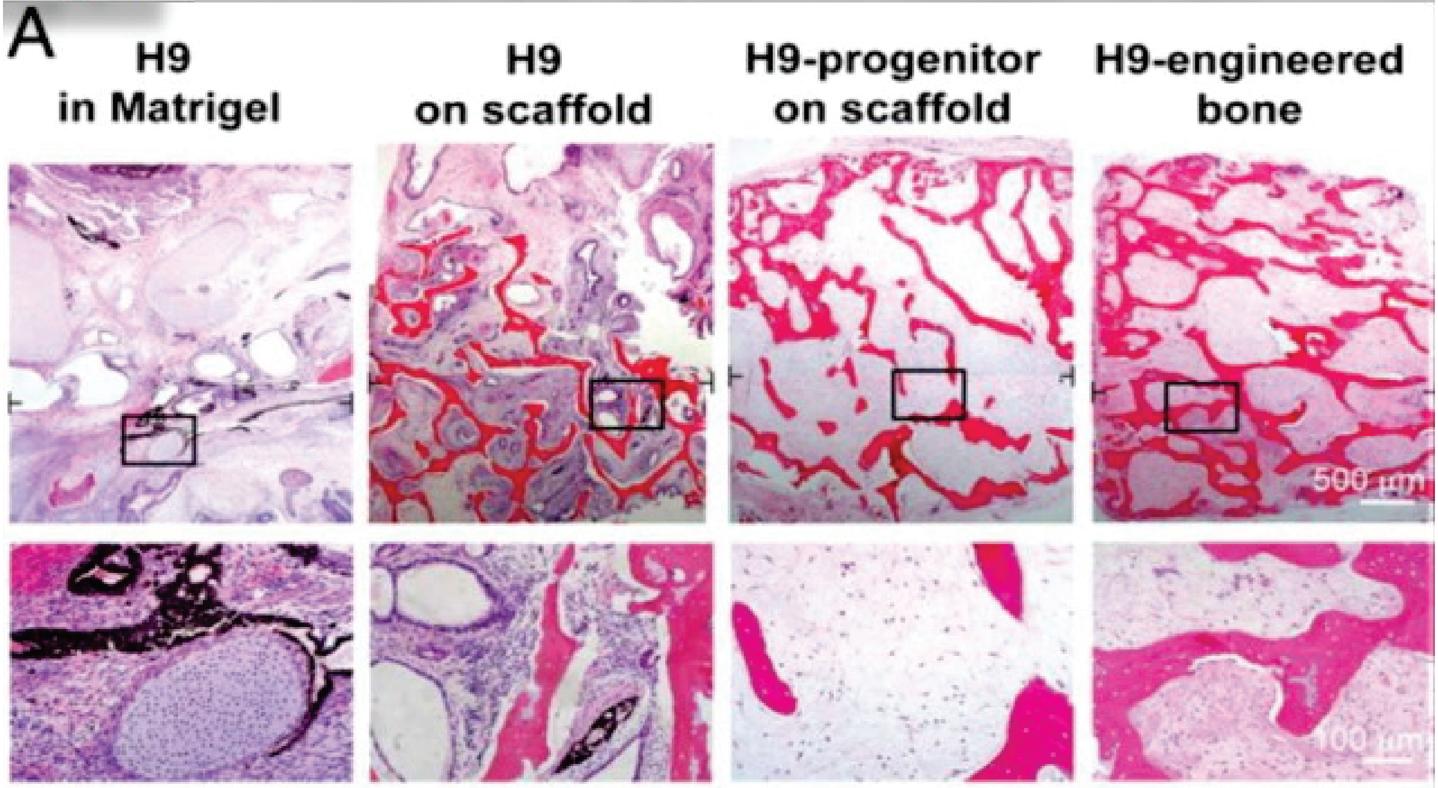


Fig 3: uCT analysis revealing bone mineralization and maturation during in vitro and invivo conditions.



Aim4: *In vivo* Safety and stability analysis of the engineered bone construct.

Fig 4



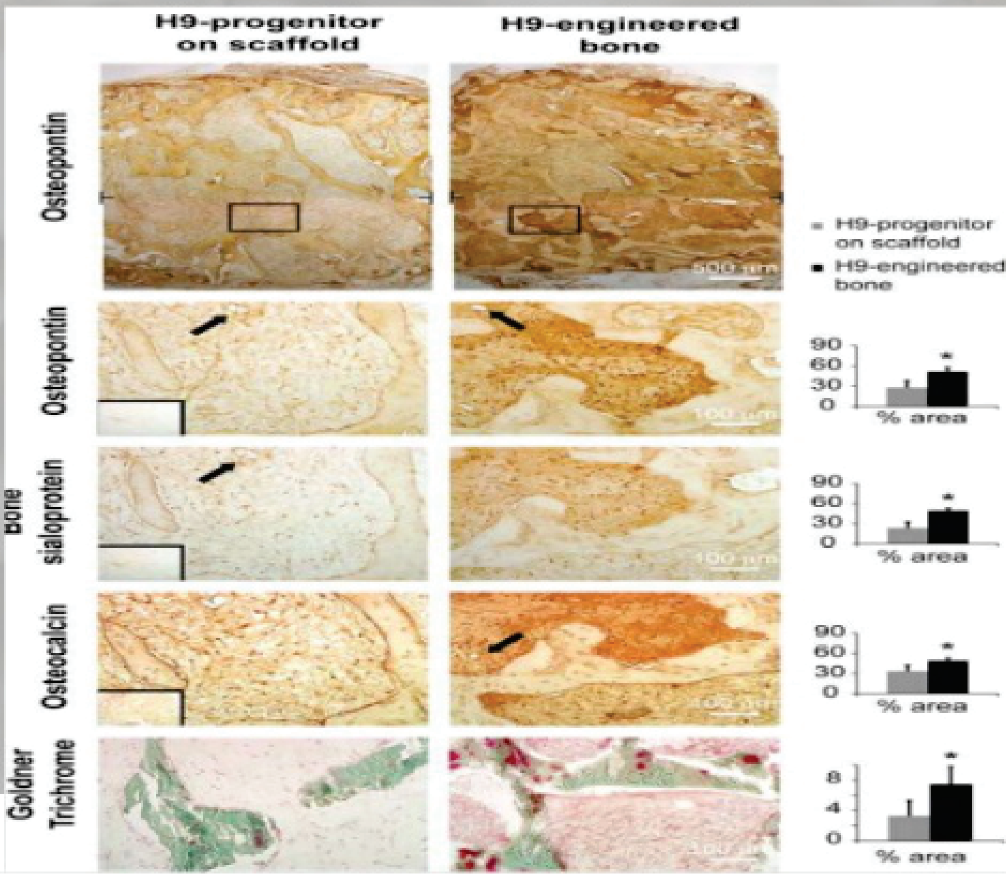


Fig 4B

And what am I proposing..

- Analysis of Bone-tissue characteristics of hESC-mesenchymal progenitors generated from different tissue engineering protocols
  - Suggesting addition of Beta glycerol phosphate
  - And Ascorbic acid – 2 - phosphate.
- Determination of influence of PRGF- Endoret implanted 3D scaffold system on the rate of development of MPCs into bone tissue.
- Long term safety studies in orthotopic implantation models.

Thank you