Bone Tissue Engineering by Bioreactor

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Q: clinical relevant?

Grafts for bone defect

Q: clinical relevant?

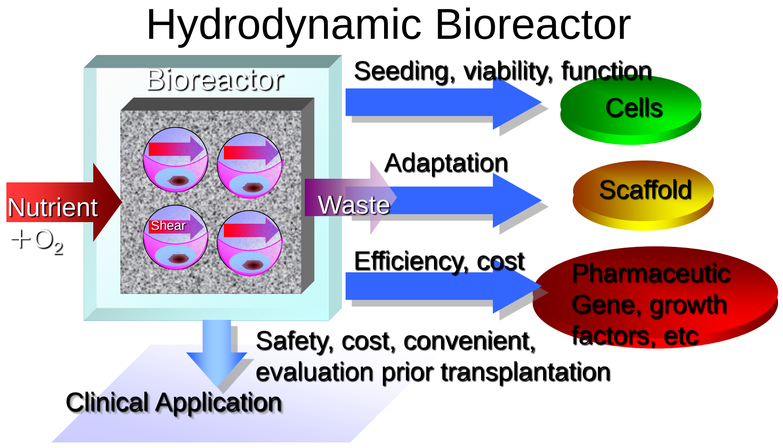
Bone regeneration in vitro

2D study by flow chamber

Oscillatory flow by mechanical loading

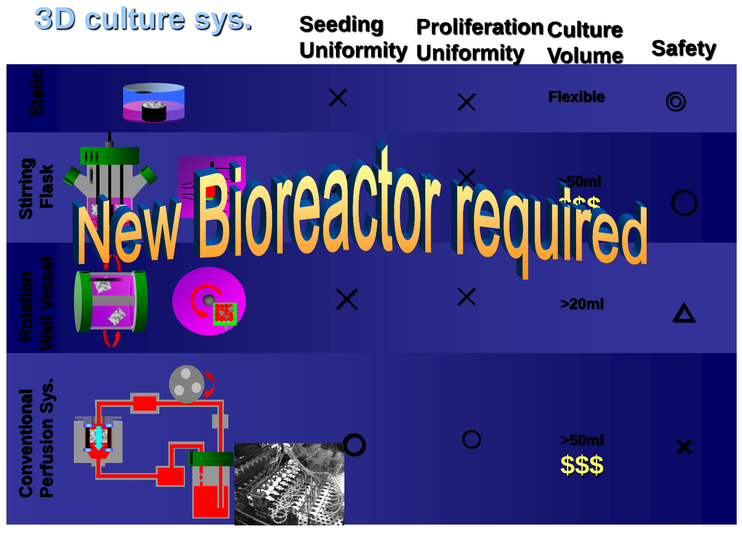
- Intracellular calcium release↑ IP_3↑, cAMP↑
- COX-2↑,, Erk-1/2 ↑ G proteins↑
- PGE_2 ↑, PGI_2↑, NO↑
- Fluid forces > Mechanical strain
- ALP activity↑ osteocalcin↑
- Osteopontin↑(2 dyn/cm²)
- Mineralized matrix deposition↑

Hydrodynamic Bioreactor



Q: what new?

3D culture sys.



Purpose

Oscillatory flow

→ Loading induced flow profile in vivo

Develop a hydrodynamic bioreactor :

Compact

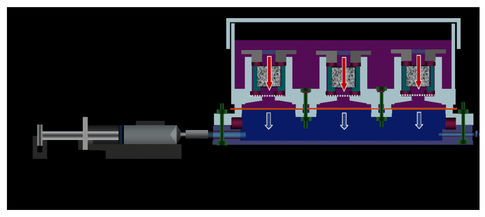
Safe

Easy operation

In-line seeding with high efficiency

Small culture volume ←→ large culture volume

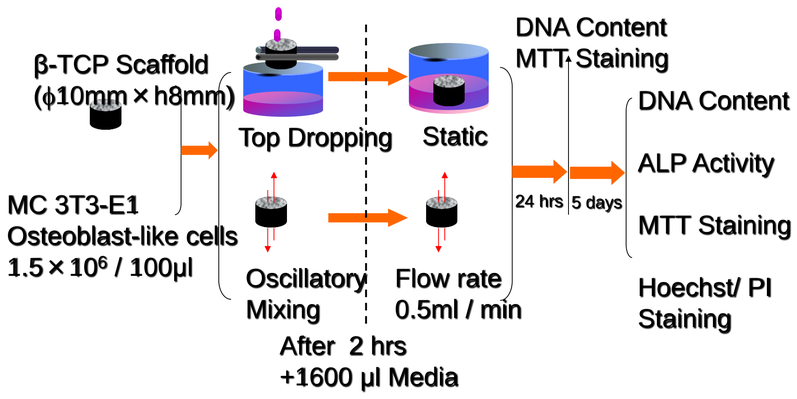
Oscillatory Perfusion System



Seeding ring

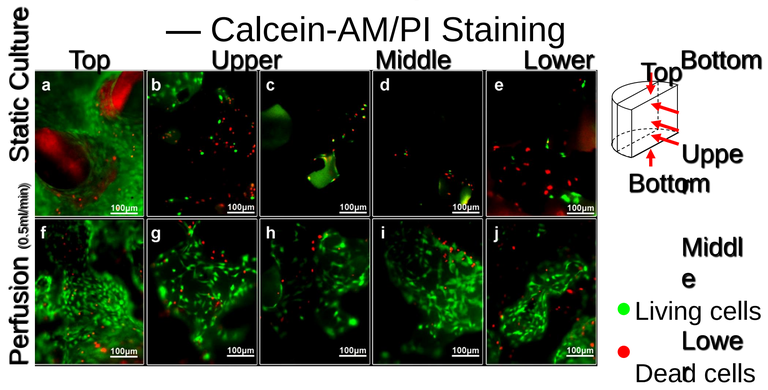
In incubator **In clean bench**

Methods of Seeding and Culture

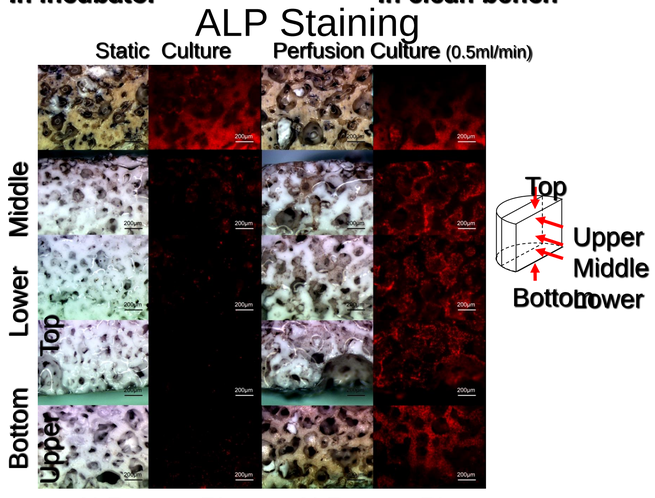


Spacial Distribution of Cell Viability

— Calcein-AM/PI Staining

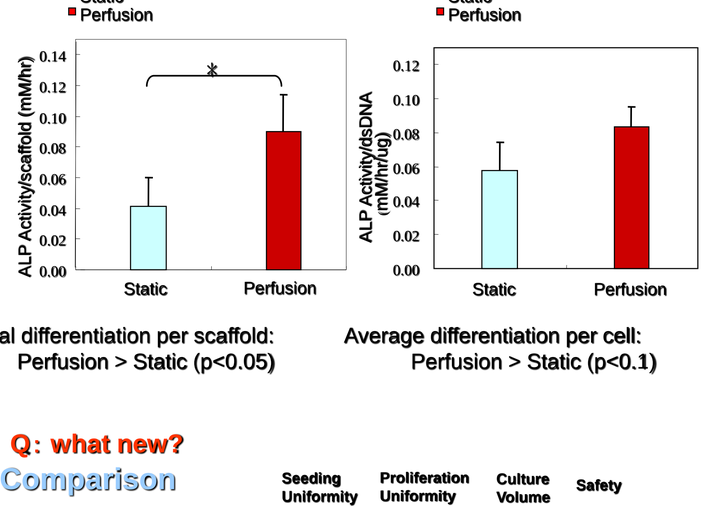


ALP Staining



ALP Activity

— Early Osteogenic Marker

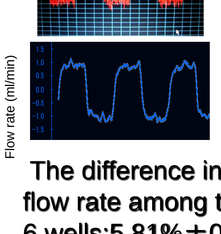


Total differentiation per scaffold: Perfusion > Static (p<0.05)

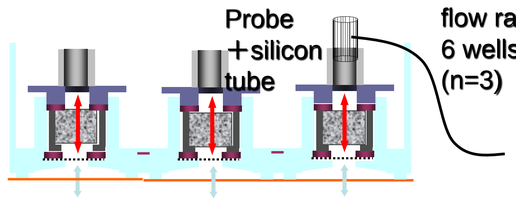
Average differentiation per cell: Perfusion > Static (p<0.1)

Evaluation of Flow Rate Waveform

RATE (ml/min/well)	Volume (ml/well)	*f* (Hz)	τ (dyn/cm²)
0.00	0	0	0
0.05	0.5	1/1200	0.004
0.50	0.5	1/120	0.04
1.00	1.0	1/120	0.08
12.00	0.2	1/2	0.96 ≈1.0
24.00	0.4	1/2	1.92 ≈2.0



The difference in flow rate among the 6 wells:5.81%±0.6 (n=3)



Spacial Distribution of Cell Viability

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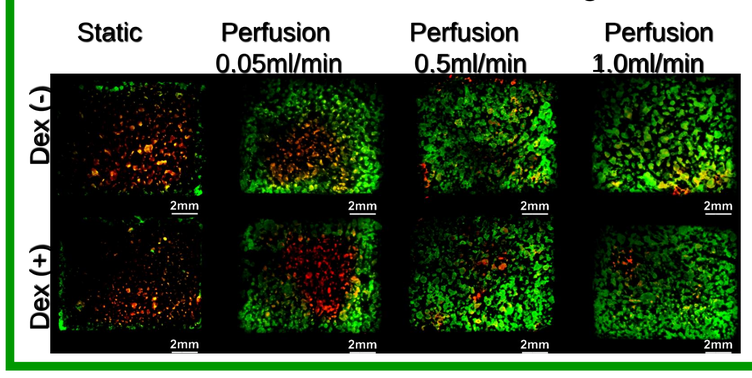
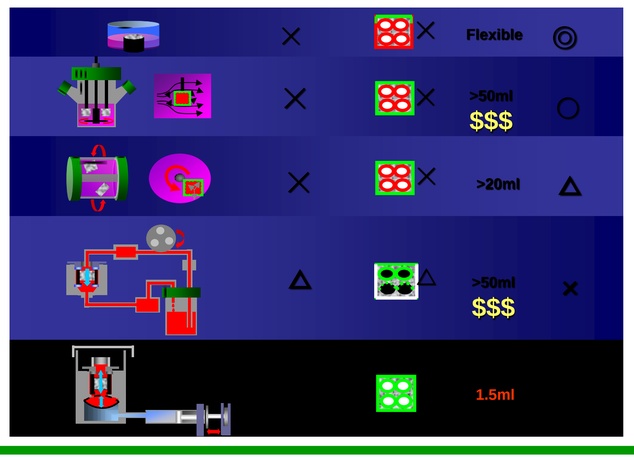


Q: what new?

Comparison



Conclusion

Tissue engineering bone with clinical relevant size could be cultured uniformly in only 1.5ml media by the oscillatory perfusion system.

Oscillatory perfusion system:

Compact, efficient seeding & culture, safe, etc.

The only bioreactor → uniform 3D culture

0.5ml/min→ optimized

Cassette design → internal flow+ external flow

Strategy of 3D culture of customized tissue engineering bone was established - first study