

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

- Intracellular calcium release \uparrow IP_3 , $cAMP$ \uparrow
- COX-2 \uparrow , Erk-1/2 \uparrow
- G proteins \uparrow
- PGE₂ \uparrow , PGI₂ \uparrow , NO \uparrow
- Fluid forces \rightarrow Mechanical strain
- ALP activity \uparrow , osteocalcin \uparrow
- Osteopontin \uparrow (2 dyn/cm²)
- Mineralized matrix deposition \uparrow

Oscillatory flow by mechanical loading

Hydrodynamic Bioreactor

Q: what new?
3D culture sys.

	Seeding Uniformity	Proliferation Uniformity	Culture Volume	Safety
Static	×	×	Flexible	⊙
Stirring Flask	×	×	>50ml	○
Rotation Well Vessel	×	×	>20ml	△
Conventional Perfusion Sys.	×	×	>50ml \$\$\$	×

New Bioreactor required

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

Methods of Seeding and Culture

β-TCP Scaffold (φ10mm × h8mm)

MC 3T3-E1 Osteoblast-like cells 1.5 × 10⁶ / 100μl

Top Dropping vs. Oscillatory Mixing (Flow rate 0.5ml/min, After 2 hrs +1600 μl Media)

Static vs. Perfusion (Flow rate 0.5ml/min)

24 hrs 5 days

DNA Content, MTT-Staining, ALP Activity, MTT Staining, Hoechst/PI Staining

ALP Activity

■ Static ■ Perfusion

— Early Osteogenic Marker

■ Static ■ Perfusion

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

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

Spacial Distribution of Cell Viability

— Calcein-AM/PI Staining

Top Middle Lower

Upper Middle Lower

Bottom Upper

● Living cells ● Dead cells

Q: what new?
Comparison

	Seeding Uniformity	Proliferation Uniformity	Culture Volume	Safety
Static	×	×	Flexible	⊙
Stirring Flask	×	×	>50ml \$\$\$	○
Rotation Well Vessel	×	×	>20ml	△
Conventional Perfusion Sys.	×	×	>50ml \$\$\$	×
Oscillatory Perfusion System	⊙	⊙	1.5ml	⊙

ALP Staining

In incubator: Static Culture, Perfusion Culture (0.5ml/min)

In clean bench: Perfusion Culture (0.05ml/min), Perfusion Culture (0.5ml/min), Perfusion Culture (1.0ml/min)

ALP PI ALP PI

Evaluation of Flow Rate

Waveform

RATE (ml/min/well)	Volume (ml/well)	f (Hz)	(dyn/cm ²)
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

— Calcein-AM/PI Staining

Static Perfusion 0.05ml/min Perfusion 0.5ml/min Perfusion 1.0ml/min

Dex (-) 2mm 2mm 2mm 2mm

Dex (+) 2mm 2mm 2mm 2mm

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**