

て軟骨基質の産生の上昇が認められた。

以上より、US は骨芽細胞に対しては直接作用せず、軟骨細胞に作用し、内軟骨性骨化を促進する可能性が示された。

生体組織は細胞と細胞外基質から構成されており、細胞外基質は細胞の増殖や分化などの機能発現に重要な役割を果たしていることが報告されている。従って、人工細胞外基質として多糖類からなる PEC を作製し、PEC 上で骨芽細胞を培養し、US を照射したところ、分化を促進させる基質上で培養した MC3T3-E1 cells はさらに分化の促進が観察された。また、PEC コーティング dish の表面物性を検討した結果、アニオン性多糖類の官能基の違いにより、表面物性に差が認められた。PEC コーティング上で培養した骨芽細胞の US 照射影響が PEC の官能基の違いによって差が認められたことより、物性の違い、つまり、基質の性質の違いにより US の効果か異なることが示された。以上のことより、US の骨折治癒促進効果は骨芽細胞単独ではなく、細胞周囲の細胞外マトリクスを含めた組織に対して効果を及ぼす、つまり、人工細胞外基質の存在が重要であることが示された。

E 結論

US の骨系細胞に対する作用は、軟骨細胞に対しては細胞に直接作用するが、骨芽細胞においては

細胞に直接作用するのではなく、基質を介して作用することが明かとなった。

参考文献

- 1) Javad Parvizi et al Journal of Orthopaedic Research 17 488-494 The Journal of Bone and Joint Surgery, Inc 1999
- 2) Tong, H S et al Murine osteoclasts and spleen cell polykaryons are distinguished by mRNA phenotyping J Bone Miner Res, 9 577-584, 1994
- 3) P C SCHILLER, et al Gap-junctional Communication Is Required for the Maturation Process of Osteoblastic Cells in Culture, Bone 2001, 28 362-369

F 研究発表

- 1 論文発表
なし
- 2 学会発表

超音波照射条件下の骨芽細胞の培養と機能発現、
平田 智子、長幡操、寺本彰、阿部康次、中土幸男、
第 52 回高分子学会年次大会発表予定

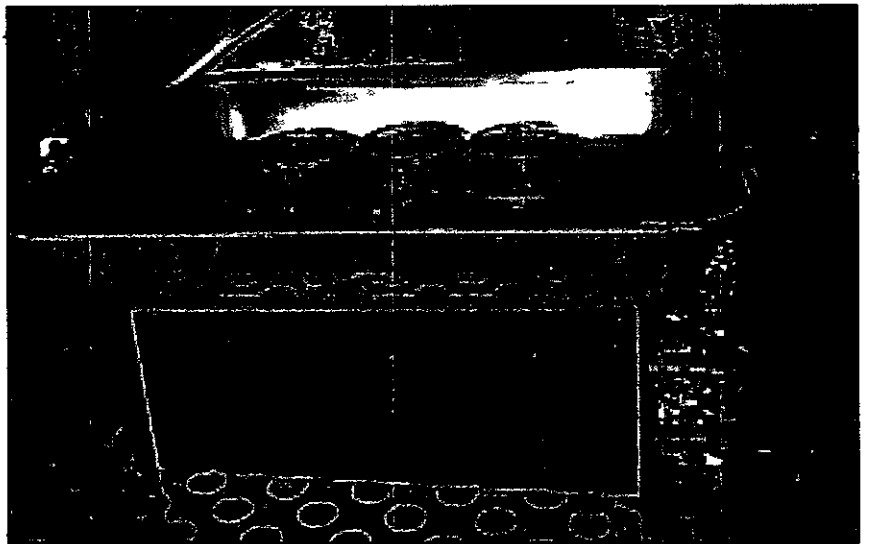
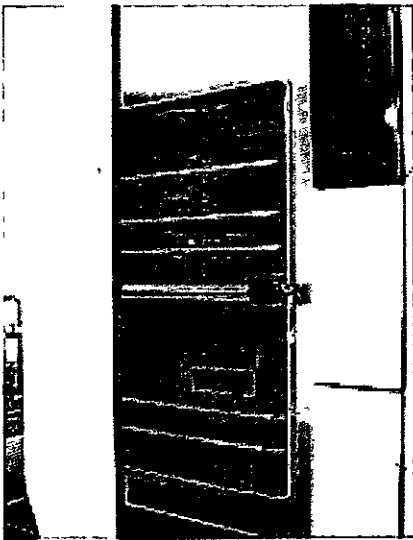
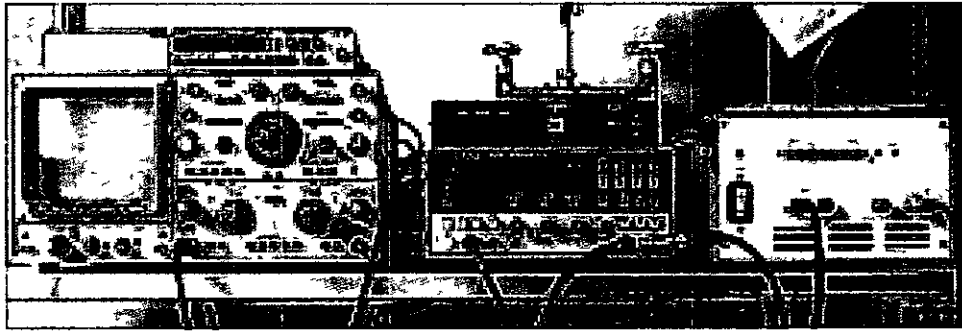


Fig.1 超音波発信装置

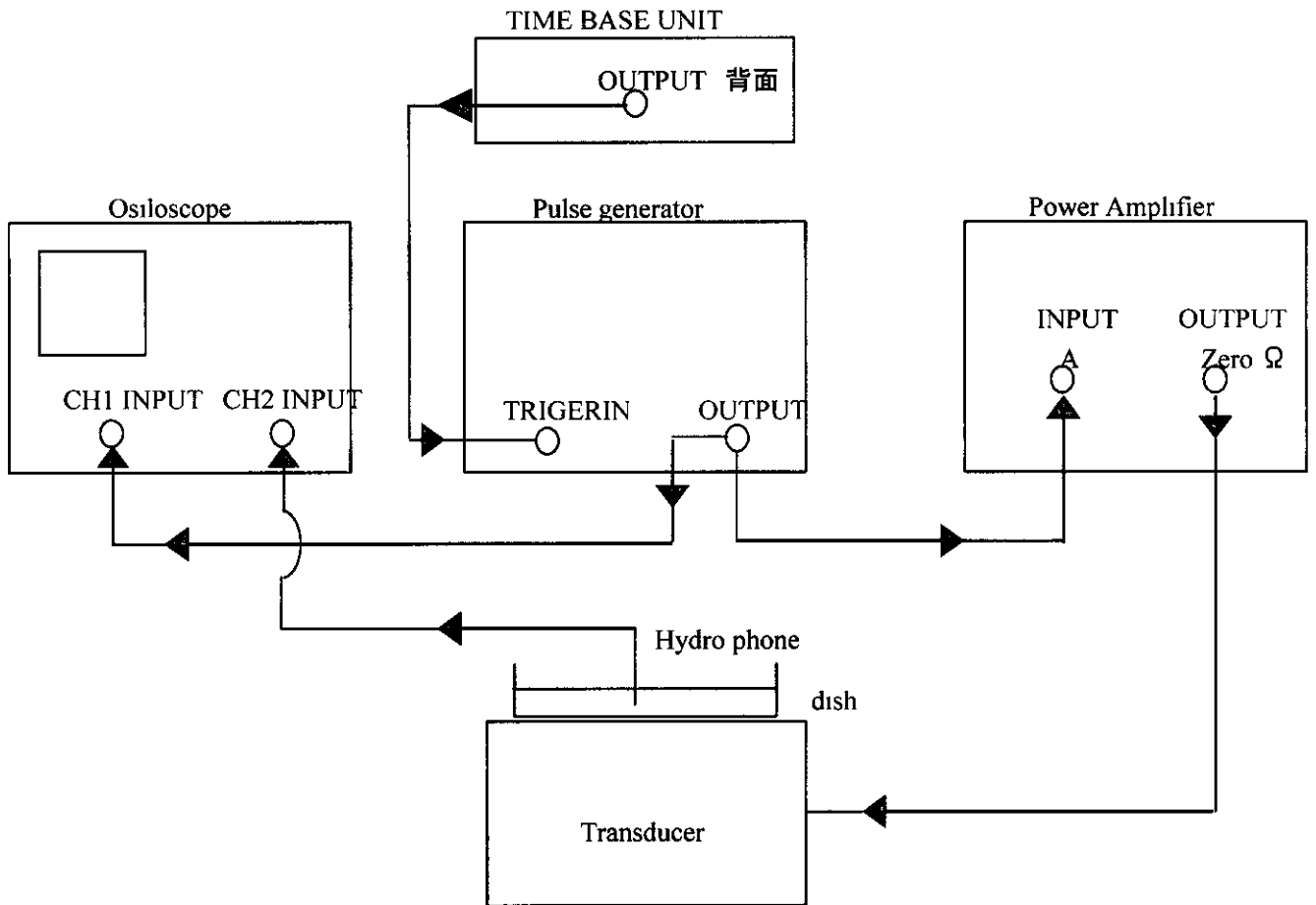
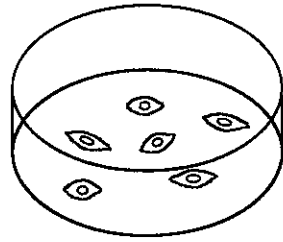


Fig.2 細胞培養用超音波発信装置のブロックダイアグラム



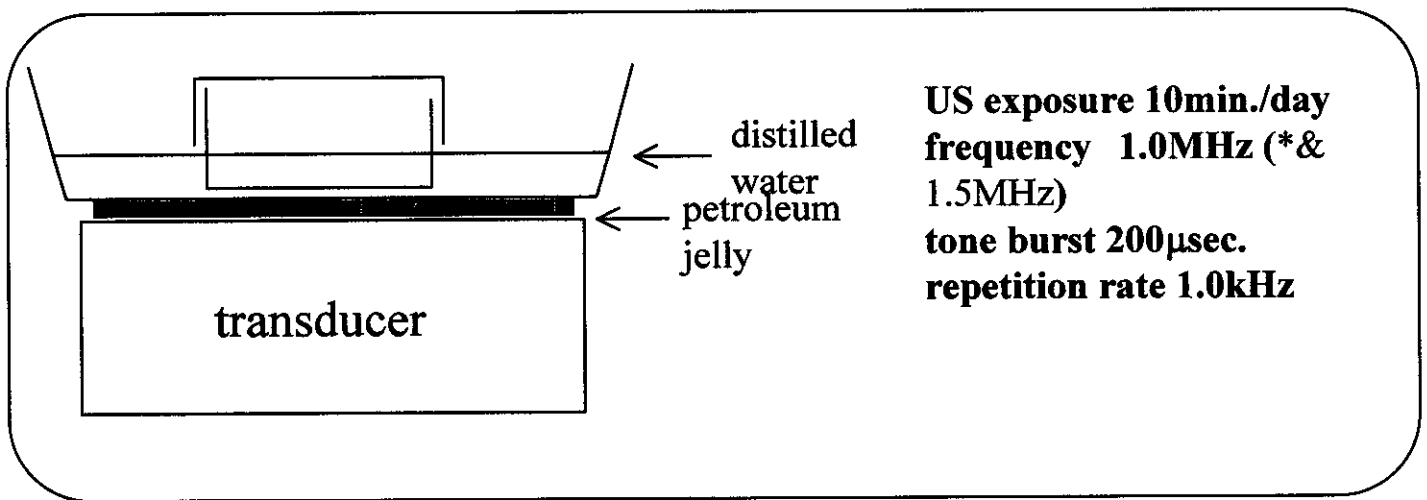
MC3T3-E1

α -MEM + 10%FBS

5×10^4 cells/35mm dish

(*Cytotoxicity :

10×10^4 cells/35mm dish)



- Cytotoxicity (LDH assay)
- Cell morphology
- Proliferation (Protein assay)
- Differentiation (ALPase activity, RT-PCR)

Fig.3 Experimental flow chart

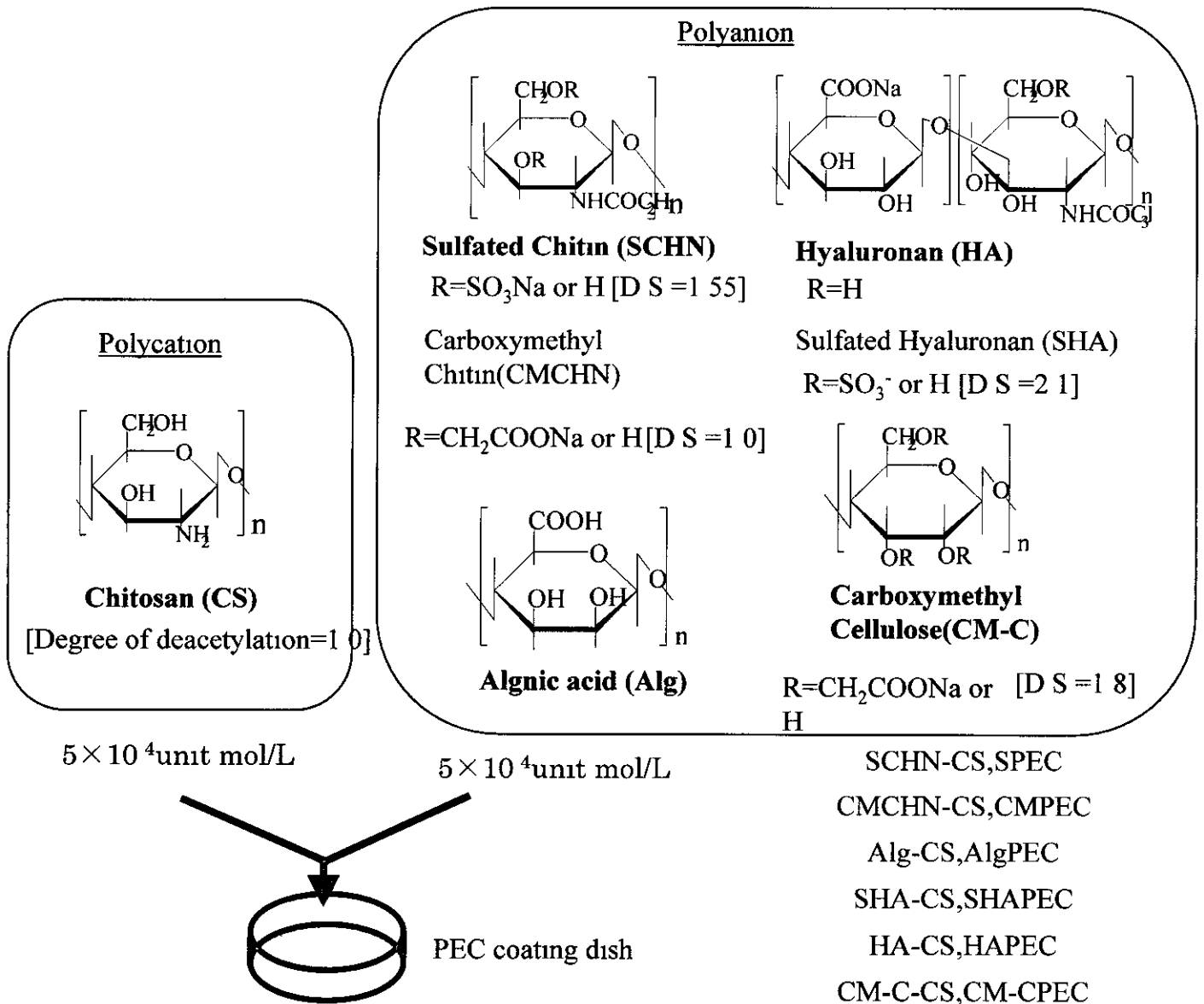


Fig.4 Experimental flow chart

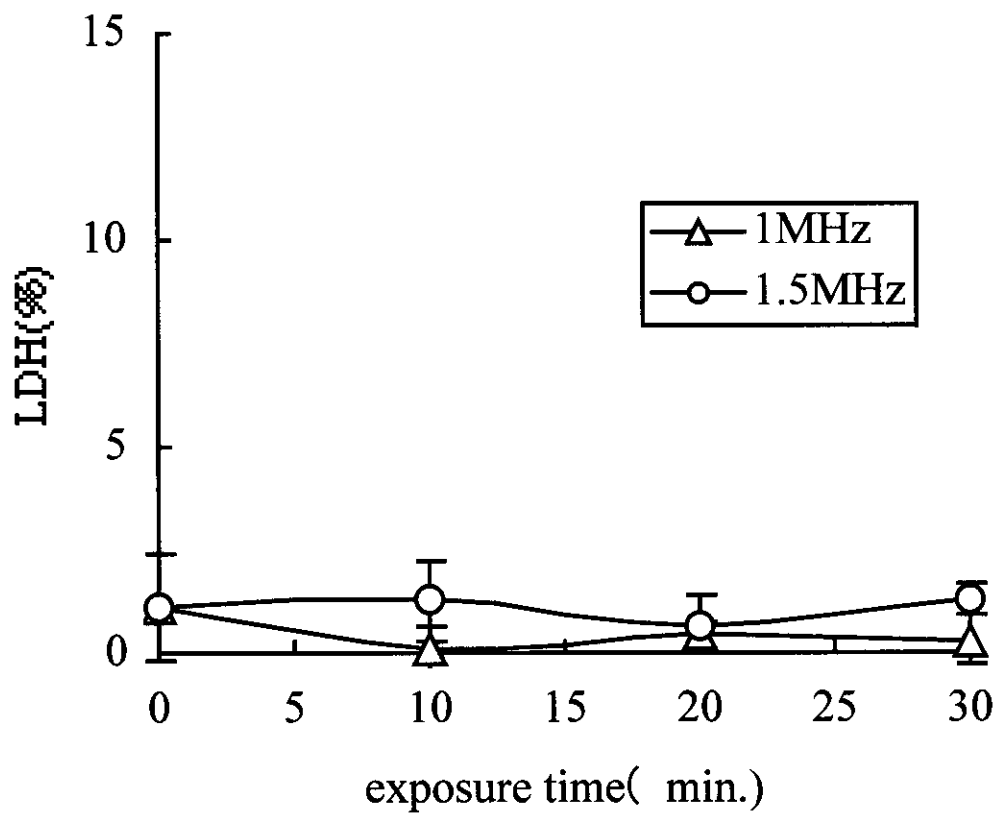


Fig. 5 Cytotoxicity results of MC3T3-E1 cells following exposure to ultrasound

TCD

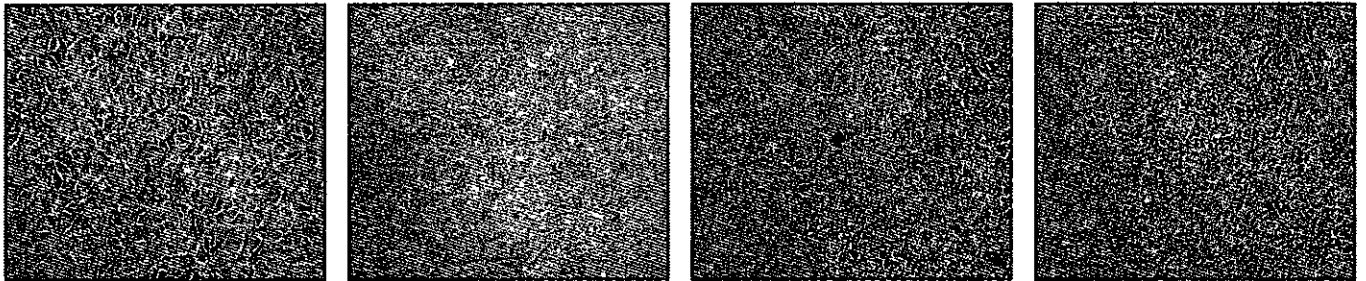
3days

7days

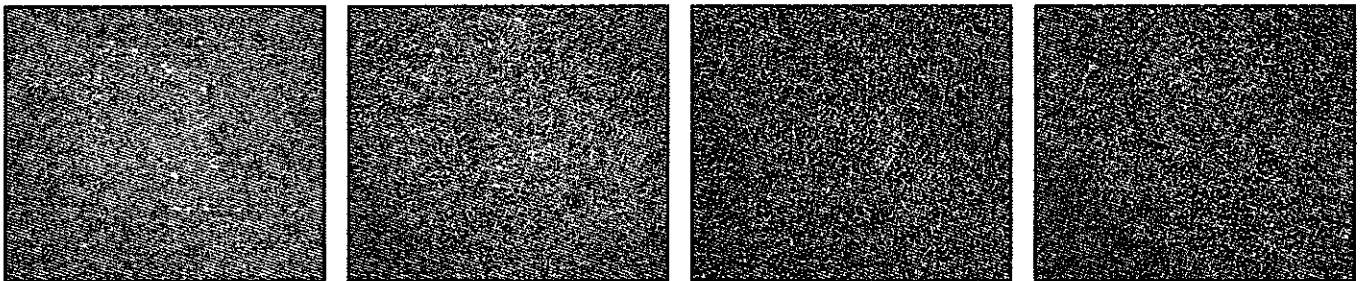
14days

21days

non-exposure



exposure



$\overline{200\mu\text{m}}$

Fig.7 Morphology of MC3T3-E1 cells cultured on TCD following exposure to ultrasound

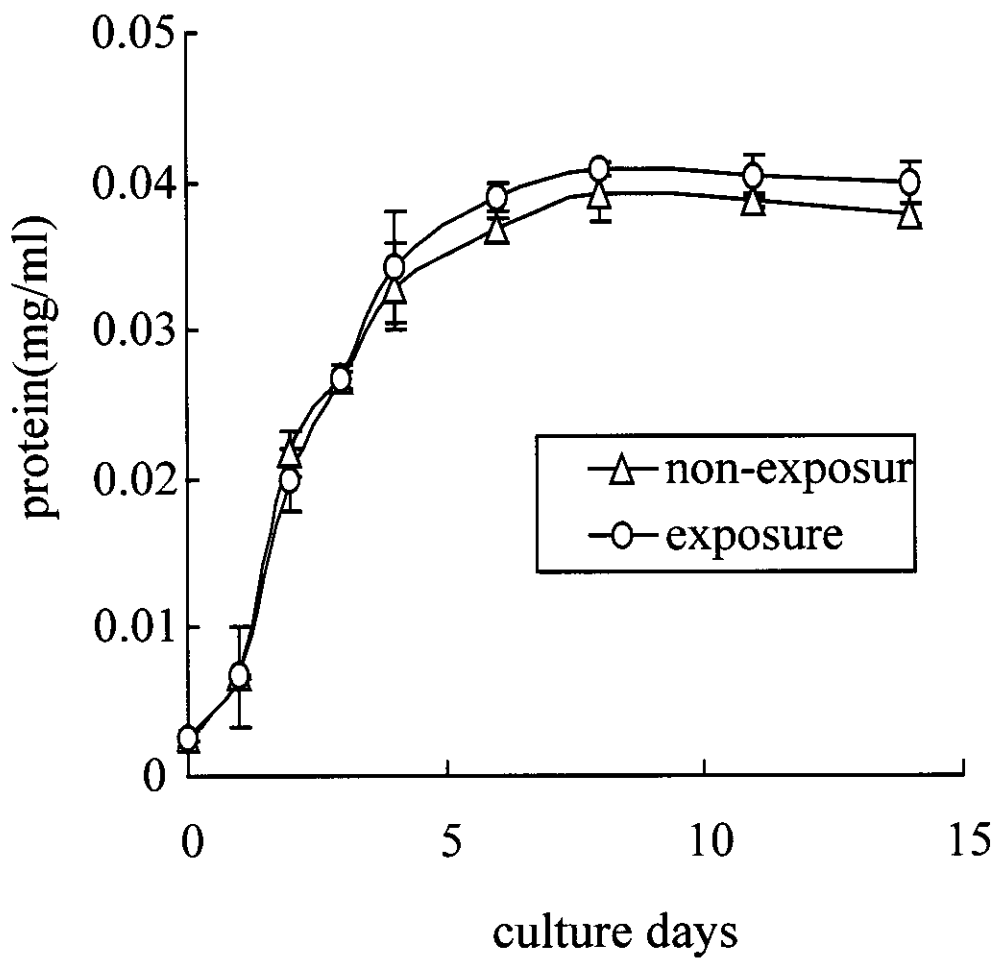


Fig.6 Proliferation of MC3T3-E1 cells following exposure to ultrasound

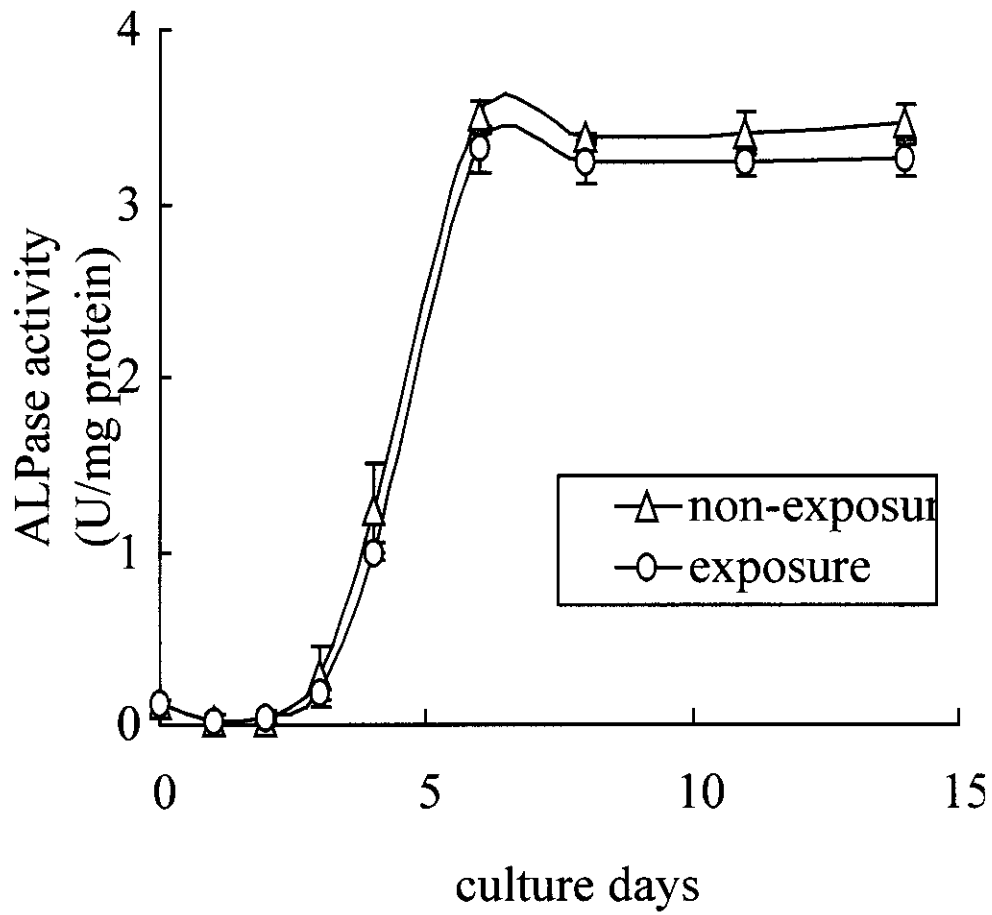


Fig. 8 ALPase activity of MC3T3-E1 cells following exposure to ultrasound

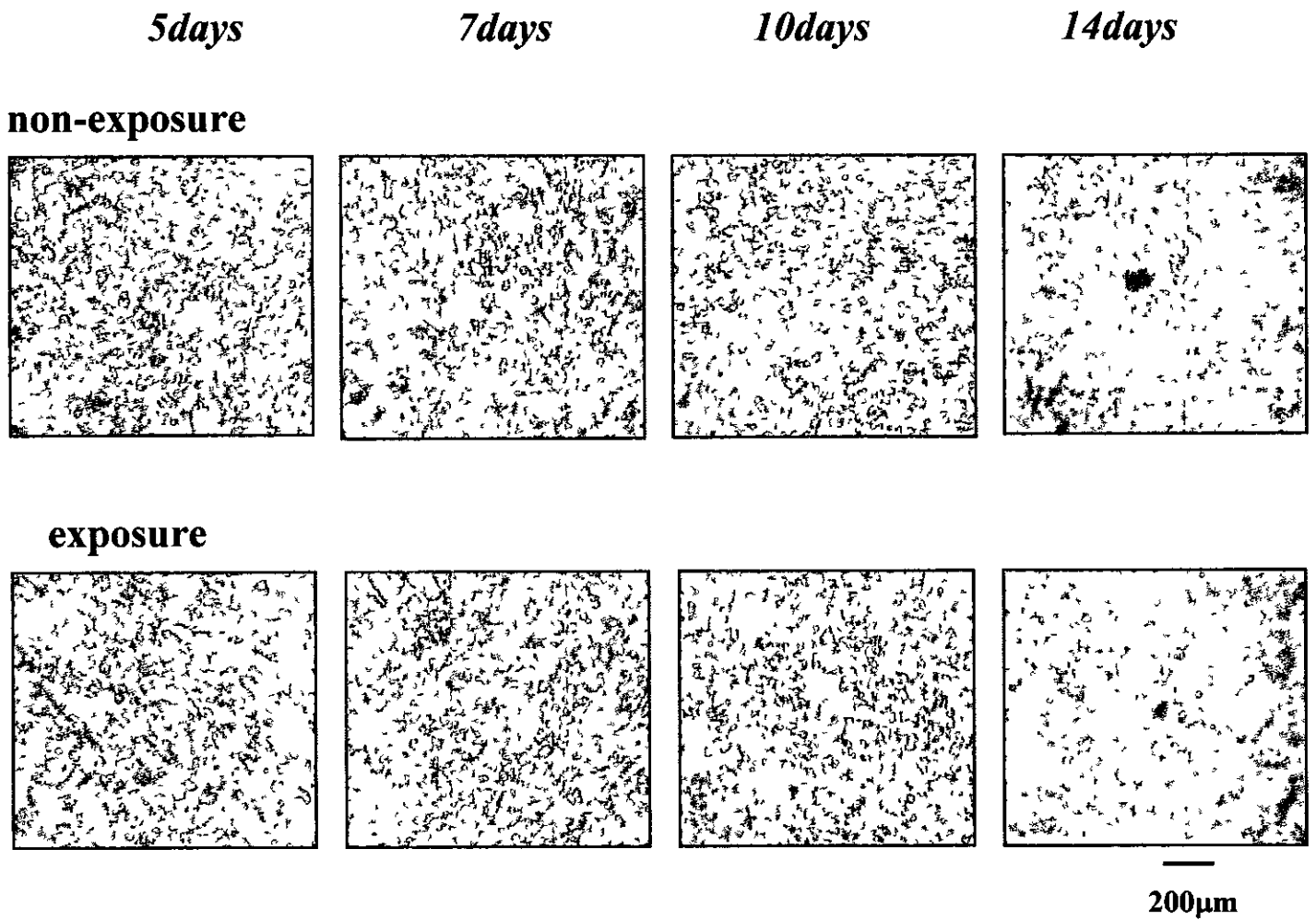


Fig.9 Azo staining of MC3T3-E1cells following exposure to ultrasound

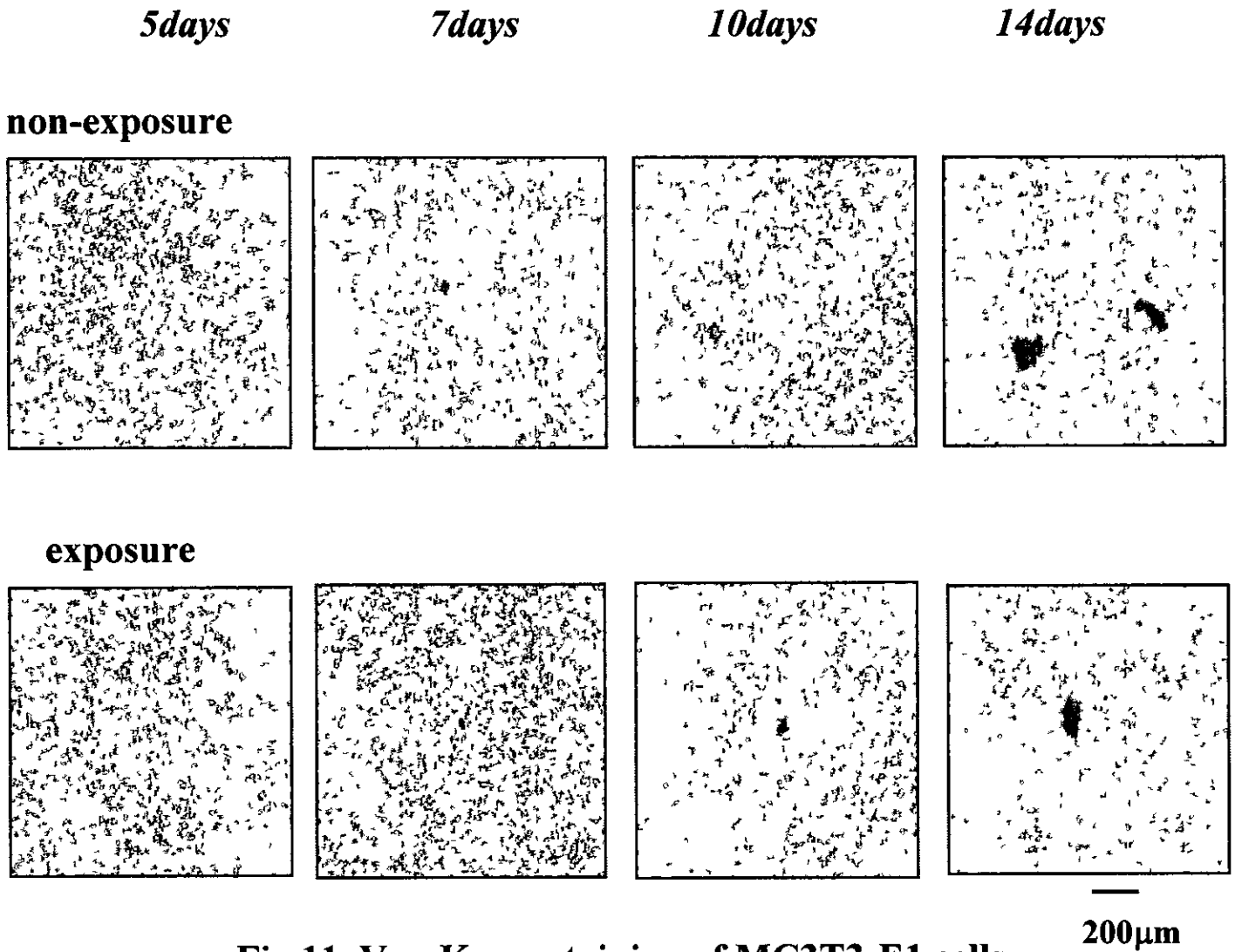


Fig.11 Von Kossa staining of MC3T3-E1 cells following exposure to ultrasound

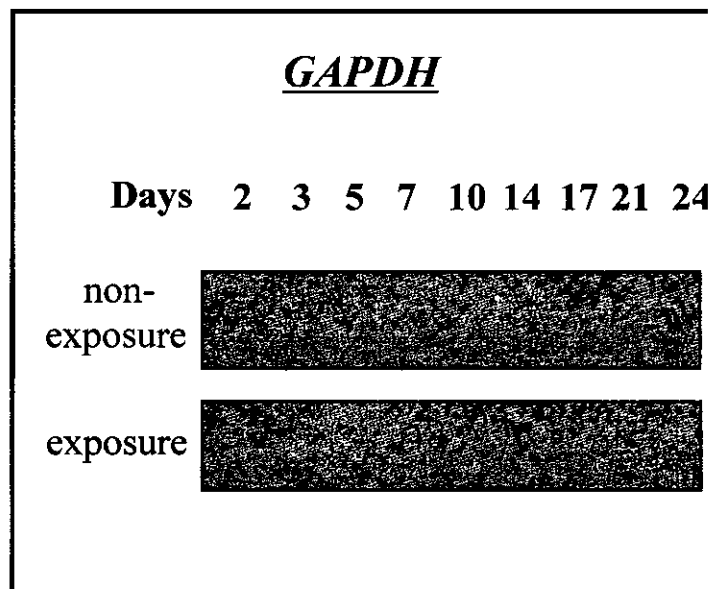
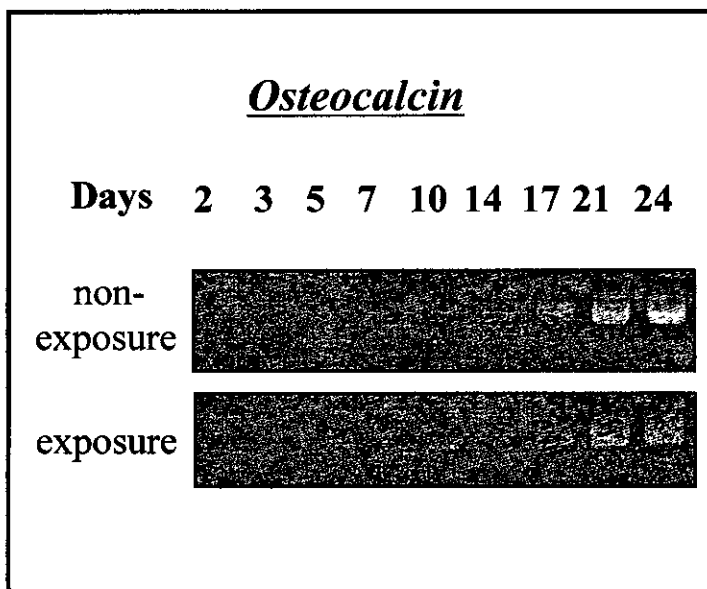
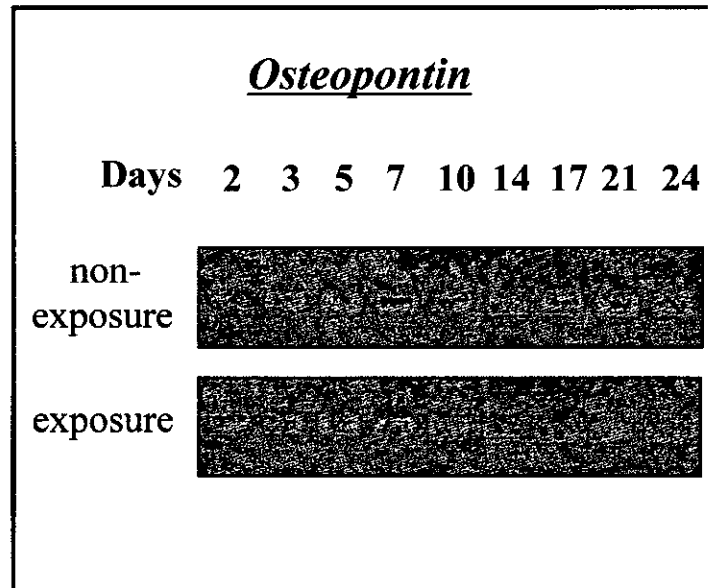
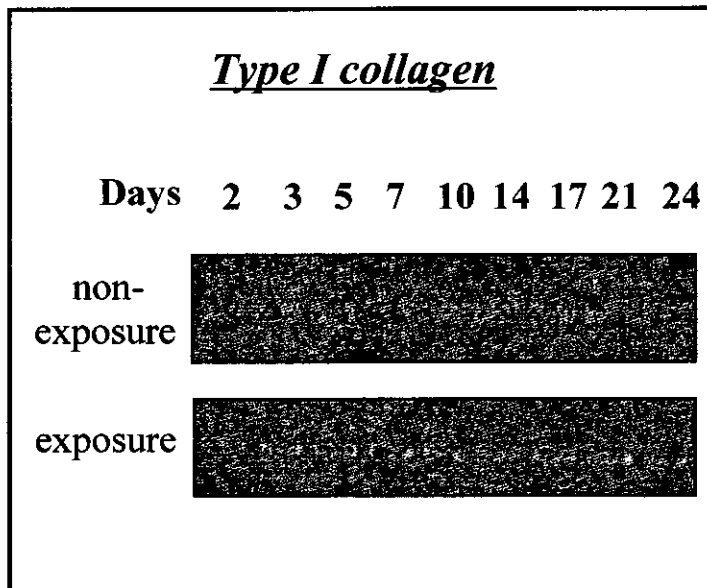


Fig.12 PCR products of MC3T3-E1 cells following exposure to ultrasound, using primers for type I collagen, osteopontin, osteocalcin mRNA

CMPEC

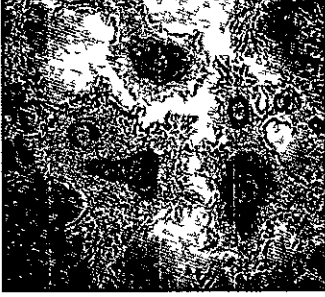
3days

7days

14days

21days

non-exposure



exposure

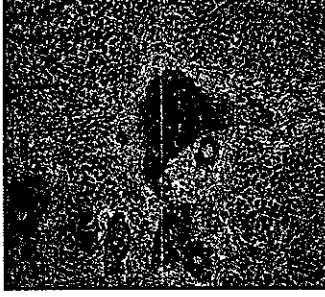
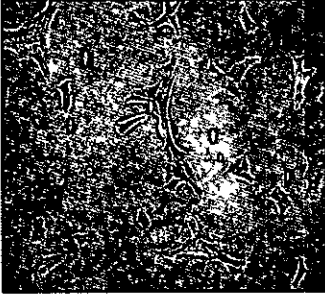


Fig.13 Morphology of MC3T3-E1 cells cultured on CMPEC following exposure to ultrasound

—
200µm

AlgPEC

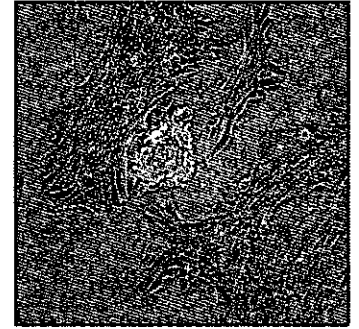
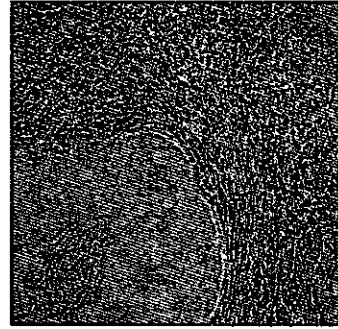
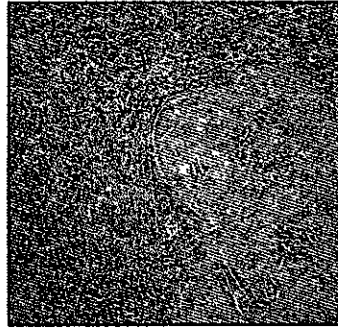
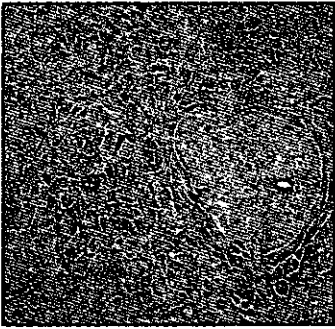
3days

7days

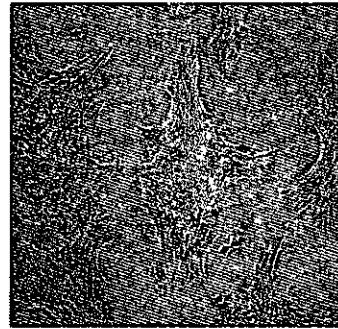
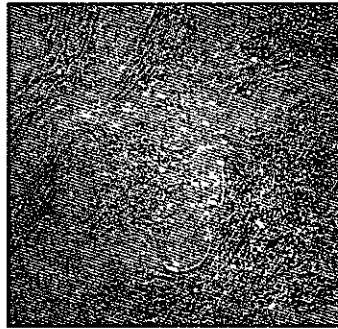
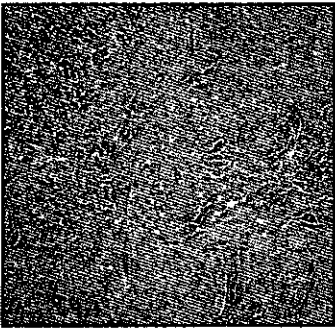
14days

21days

non-exposure



exposure



200 μ m

Fig.14 Morphology of MC3T3-E1 cells cultured on AlgPEC following exposure to ultrasound

SPEC

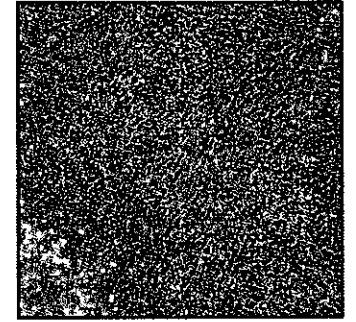
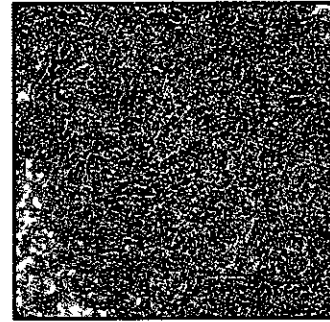
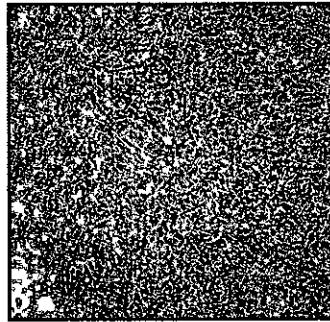
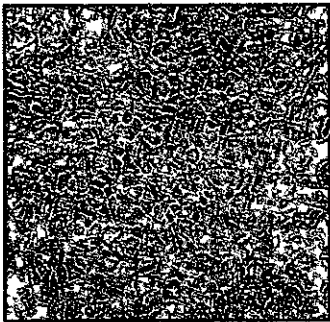
3days

7days

14days

21days

non-exposure



exposure

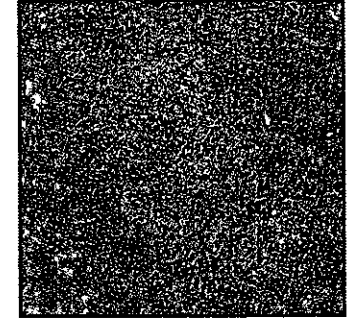
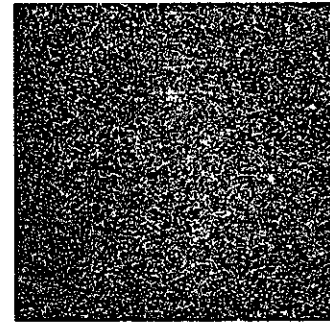
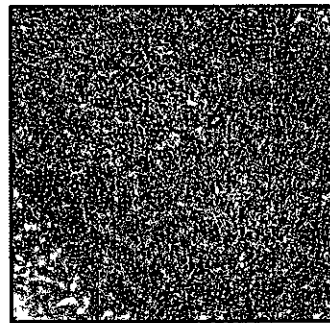
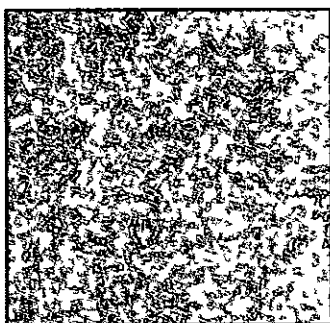


Fig.15 Morphology of MC3T3-E1 cells cultured on CMPEC following exposure to ultrasound

—
200 μ m

SHAPEC

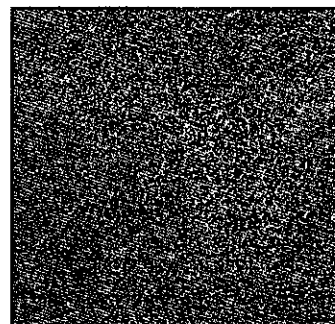
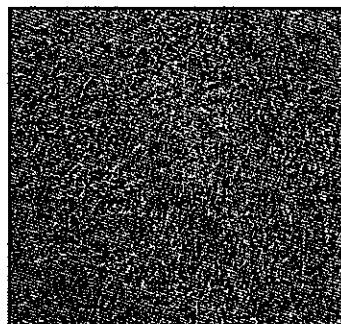
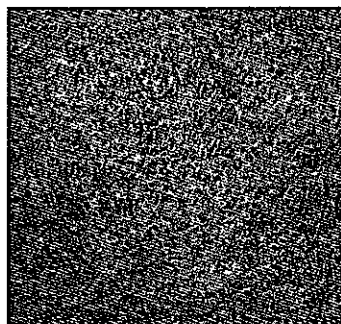
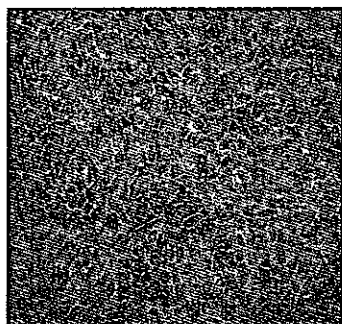
3days

7days

14days

21days

non-exposure



exposure

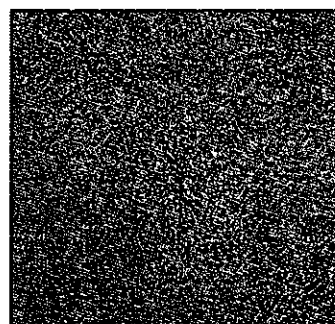
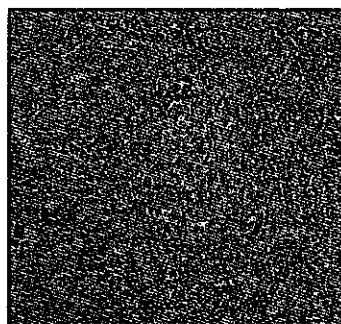
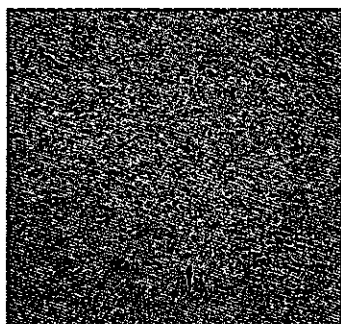
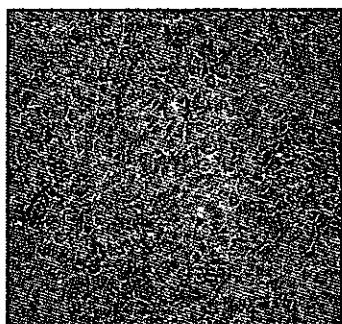


Fig.16 Morphology of MC3T3-E1 cells cultured on SHAPEC following exposure to ultrasound

—
200µm

HAPEC

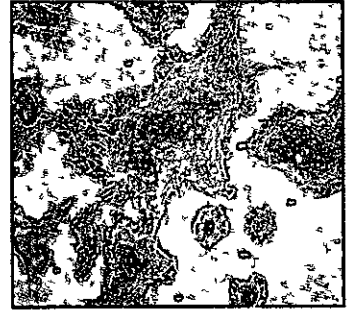
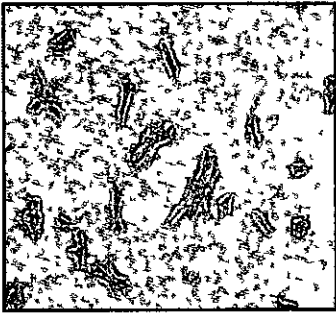
3days

7days

14days

21days

non-exposure



exposure

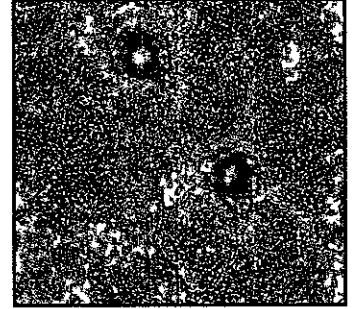
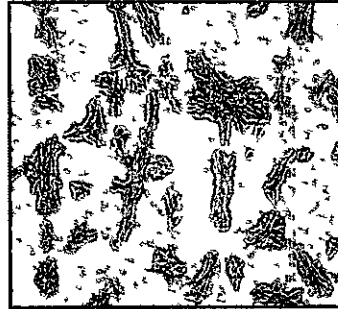


Fig.17 Morphology of MC3T3-E1 cells cultured on HAPEC following exposure to ultrasound

—
200 μ m

CM-CPEC

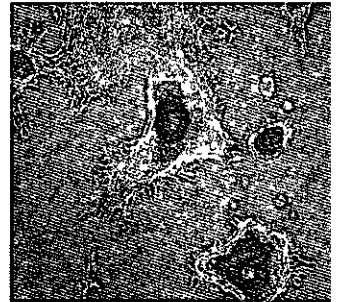
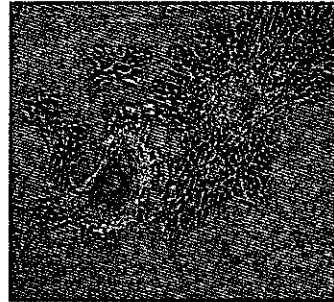
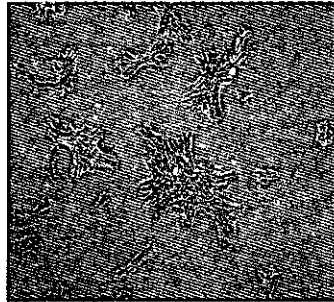
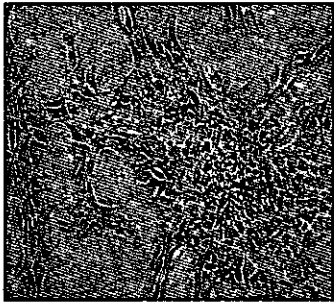
3days

7days

14days

21days

non-exposure



exposure

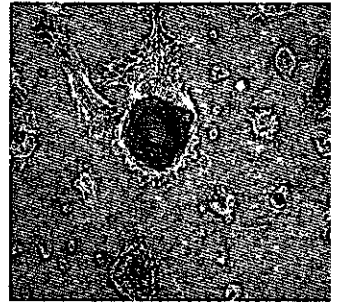
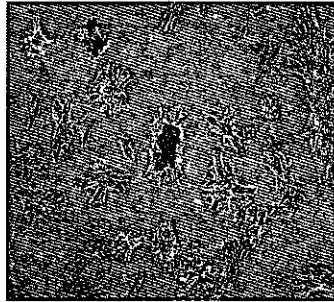
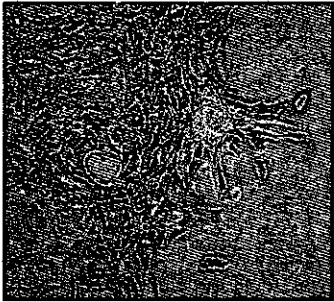


Fig.18 Morphology of MC3T3-E1 cells cultured on CM-CPEC following exposure to ultrasound

—
200 μ m