

Influences of buffer systems on chondrocyte growth during long-term culture in alginate

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INTRODUCTION: Chondrocyte behaviour is very sensitive to culture environment such as physical and biochemical conditions. To determine the influence of buffer systems on chondrocyte fate during long-term culture, HEPES buffered media in the absence and the presence of bicarbonate were used, respectively.

METHODS: Bovine articular chondrocytes were cultured in 1.2 % alginate beads for up to 12 days, at the density of 4 million cells/ml. Culture medium A was DMEM buffered by with HEPES (25 mM). Culture medium B had the same compositions as Culture medium A, but buffered by a combination of NaHCO₃ (44 mM) and HEPES (25 mM). The pH was adjusted to pH 7.4 and the osmolarity of both culture media was adjusted to 380 mOsm. The alginate beads were cultured in 24-well microplate (3 beads/well) with 2 ml culture medium A in a humidified air incubator and 2 ml culture medium B in a 5% CO₂ humidified incubator at 37°C up to 12 days, respectively. The culture medium was replaced every 2-3 days. Cell density was measured by DNA content using Hoechst 33258. Intracellular pH, glycosaminoglycan (GAG) and collagen production were measured at day 5 and day 12. Cell morphology, distribution and viability in alginate beads were monitored using multiphoton microscope over 12 days of culture.

RESULTS: The cell density in the presence of NaHCO₃ was dramatically greater than that in the absence of NaHCO₃ at the end of 12 days of culture from DNA assay and multiphoton microscope analysis (Fig. 1). In the presence of bicarbonate, the intracellular pH was more alkaline, about 0.2 pH unit (Fig. 2). Although there was no significant difference in collagen production with culture time in the presence of NaHCO₃, about 50 % more GAG was deposited in alginate beads when chondrocytes were cultured in the combination of HEPES and bicarbonate, compared to chondrocytes cultured in the absence of NaHCO₃ at the end of 12 days of culture.

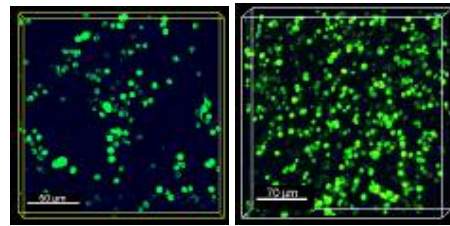


Fig. 1: Cell morphology and viability after 12 days of culture: HEPES only vs. Bicarbonate and HEPES (right).

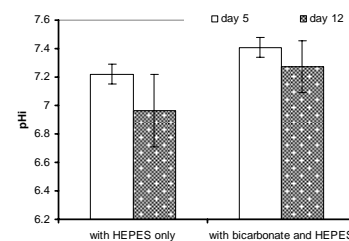


Fig. 2: Intracellular pH of bovine chondrocytes in alginate beads during 12 days of culture under HEPES only and HEPES and bicarbonate together

DISCUSSION & CONCLUSIONS: Culture medium buffered with a combination of HEPES and bicarbonate provides a relatively stable culture environment to chondrocyte seeded in the hydrogel scaffolds in the presence of CO₂. The presence of sodium bicarbonate results in more alkaline in the intracellular pH of bovine chondrocytes after long-term culture. The combination of HEPES and bicarbonate in culture medium improves cell growth, matrix production in 3D alginate beads, and more chondrocytes are grown in pairs and clusters, similar to the state in native articular cartilage.

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