

Bone Marrow Stem Cells Grown on Silastic Membranes Differentiate into Cartilage

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ABSTRACT

Our laboratory has used bone-forming cartilage from embryonic mouse limb bud cells differentiated in a rotating bioreactor to heal defects created in the skulls of mice. However, these rounded nodules were not a good fit for the defect. Consequently methods of producing a flat piece of cartilage, better suited for skull implants, were explored. In the current experiments, bone marrow mesenchymal stem cells were differentiated into cartilage on Silastic gas exchange membranes ("bubbles") developed as a hardware component for spaceflight (IML-2; 1992). **OBJECTIVE:** Our objective was to differentiate bone marrow stem cells on the membrane, producing a flat piece of cartilage. **METHODS:** Isolated bone marrow cells from 7-9 day old strain C57BL mice were expanded in culture. After 2 passages, cells suspended in medium were inoculated onto membranes supported by hardware casings or in assembled hardware units. Cultures were placed in Petri dishes and incubated at 37°C with or without 5% CO₂ for two hours; 5% CO₂ was used thereafter. After medium addition, cells were cultured for 3-7 days with ½ the medium changed every other day, then fixed with formalin. **RESULTS:** Cells with no CO₂ during the first two hours of incubation formed small aggregates and occasional larger (0.5mm) ones. Aggregates stained with alcian blue at low pH and metachromatically with Toluidine blue, indicating cartilage matrix production. Cells receiving initial CO₂ spread on the membrane, developing numerous extended processes and a neuronal appearance. Methylene blue staining revealed Nissl bodies in the cytoplasm of neuronal-like cells. **CONCLUSION:** The initial gas environment coupled with the Silastic substrate influenced cellular differentiation, but the desired shape was realized only in membranes in casings. In future studies, TGF-β will be used to produce a larger piece of tissue.