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EGGSHELL MEMBRANES AS A NATURAL SCAFFOLD FOR CELL ATACHMENT, PROLIFERATION AND DIFFERENTIATION

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The avian eggshell is a highly ordered natural acellular composite bioceramic in which the inorganic moiety is formed in conjunction with organic biopolymers in the form of proteins and proteoglycans. It is a highly ordered structure formed by layers of fibrillar eggshell membranes (ESM) and calcified matrix. The fibrillar eggshell membranes are mainly composed of highly cross-linked type X collagen, and have been experimentally used as an enzyme immobilization support and metals and stains adsorbent, and a template for crystal growth. We have previously used ESM as a substrate for culturing chicken primary calvaria-derived osteogenic cells. Additionally, pieces of ESM as an in vivo scaffold to regulate bone regeneration have been used. Here we explored the ability of ESM to support the culture of rat bone marrow mesenchymal stem cells (MSC) induced to differentiate to chondrocyte in a 3D scaffold. Rat bone marrow MSC cultured on sterilized ESM in DMEM Medium with or without chondrogenic inductors for 7 to 21 days at 37ºC in a cell incubator were used. Cell viability, total protein secretion, collagen II secretion, cartilage-specific matrix proteins (aggrecan and type II collagen) expression, and cell morphology were analyzed by MTT, Bradford, ELISA, immunoperoxidase and immune-beads, and scanning electron microscopy (SEM) respectively. During culture, these cells secrete to the medium almost double amount of proteins. By using monoclonal antibodies for immunolabelling cartilage-specific proteins in ESM cultured with MSC, the occurrence of type II collagen and aggrecan was observed, but only in those membranes cultured in the presence of chondrogenic inductors. SEM observation showed that in ESM cultured with MSC in the absence of chondrogenic inductors, cells spread as fibroblast-like shape on the ESM. On the other hand, spherical or cylindrical aggregates of cells located inside the eggshell membranes was observed in ESM cultured with MSC in the presence of chondrogenic inductors. There was a positive immunobeads-labelling for type II collagen and aggrecan around these aggregates. It is safe to conclude that eggshell membranes could be used as promising natural fibrillary scaffolds to produce a 3D cartilage implants. On the other hand, because eggshell membranes show some negative charge chemical groups due to ascorbic and glutamic acid residues, we are presently studying the effect of adding additional carboxyl groups to this scaffold by covalently attach polyglutamic acid chains, on neonatal skin rat fibroblasts attachment and proliferation. These preliminary results will be discussed here. (Financial support: FONDECYT N 1180734)