Evaluation of Microcarrier Formulations for Propagation of Osteoblasts and Chondrocytes

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Introduction: Retrieval of osteoblasts and chondrocytes from bone and cartilage became possible with availability of enzymes to break down the extracellular matrix without lethal damage to cells. Since a limited number of cells are obtained from the enzymatic treatment of tissue, techniques to promote cell multiplication have been developed to maximize retrieval success. One of the most common techniques to propagate cells is by monolayer culture. Although cells multiply, they undergo phenotypic changes. In particular, chondrocytes assume a fibroblast-like morphology. They shift from producing type II to type I collagen, and they decrease their synthesis of high molecular weight proteoglycans in monolayer culture. A strategy to promote chondrocyte proliferation while maintaining the original phenotype was developed by using a microcarrier spinner culture system. The present study tested the hypothesis that collagen-based microcarriers are the most suitable formulation for the propagation of osteoblasts and chondrocytes.

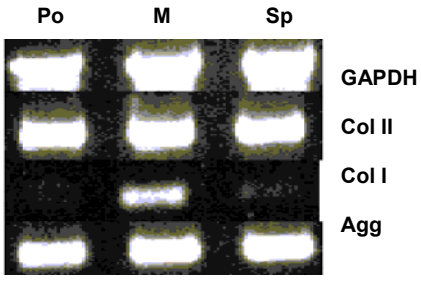
Materials and Methods: Human osteoblast-like MG-63 cells, articular and nasal chondrocytes were seeded on different microcarrier formulations[1,2]. Cells seeded onto seven different formulations of microcarriers were incubated in spinner culture at 60 rpm, 37°C, 5% CO_2 for two weeks. Cells were retrieved for analysis of phenotype expression. Transcript levels of type I and II collagen, proteoglycans, alkaline phosphatase, and osteocalcin were determined by the reverse transcription-polymerase chain reaction (RT-PCR). The S14 or GAPDH housekeeping gene was used to verify equal loading.

Results: Trabecular bone-derived osteoblasts and osteoblast-like MG-63, articular and nasal chondrocytes attached with ease and proliferated on solid collagen microcarriers (Cellagen and Inamed) as shown in the table below. Cells on these cross-linked solid collagen microcarriers appeared spherical. All cells remained viable and proliferated 5- to 20-fold within two to three weeks. Chondrocytes in spinner culture (Sp) continued to express cartilage marker Col II but not Col I, similar to those in cartilage tissue designated as P0. In contrast, the other microcarrier formulations shown in the table did not achieve the same plating efficiency and ability to promote phenotype expression as the solid collagen beads.

Discussion / Conclusion: The present study demonstrates that microcarriers composed of cross-linked solid type I collagen best supported the proliferation and function of osteoblasts and chondrocytes. Our data suggests that these cell types detect the chemical as well as topographical structure of the microcarriers.

Beads Tested	Type of Microcarriers	Cells Tested	Conclusion
Cellagen (MP Biomedicals)	Bovine Collagen Type I	Osteoblasts, MG-63, and Chondrocytes	Cells attach, proliferate, high viability (100%)
Inamed Beads (Inamed)	Bovine Collagen Type I	MG-63 and Chondrocytes	Cells attach, proliferate, high viability (100%)
C102-152 (SoloHill)	Porcine Collagen Type I-coated on polystyrene core	MG-63 and Chondrocytes	Cells moderately attach (50%)
C104-152 (SoloHill)	Porcine Collagen Type I-coated on polystyrene core	MG-63	Cells attach ed poorly (25%)
F102-152 (SoloHill)	Cationic Porcine Collagen Type I-coated on polystyrene core	MG-63	Cells attached poorly (25%)
Culti-sphere G (Hyclone)	Microporous Collagen	MG-63 and Chondrocytes	Low viability (25%), cells penetrate porous beads
Cytodex 1 (Amersham Biosciences)	Crosslinked Dextran	MG-63 and Chondrocytes	Beads break, Inconsistent cell attachment >90% viability

Figure 1: Phenotype Expression of Human Articular Chondrocytes



References:
1. Frondoza, CG. et al. J Biomaterials 17:879-888,1996.
2. Overstreet M. et al. In Vitro Cell Dev Biol Anim. 39 (5-6):228-34. 2003.

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