Chondrogenic Differentiation of Adult Mesenchymal Stem Cells and Embryonic Stem Cells

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Chondrogenic Differentiation of Adult Mesenchymal Stem Cells and Embryonic Stem Cells

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Abstract—Mesenchymal stem cell (MSC) contraction associated with chondrogenesis is attributed to the expression of α-smooth muscle actin (α-SMA). In this study, pluripotent embryonic carcinoma cells (ECCs) and MSCs were compared for cartilage histogenesis. Both cell types expressed α-SMA in monolayer. However, when cultured in pellets and in 3-D scaffolds, only MSCs contracted and formed glycosaminoglycan (GAG)- and type II collagen-rich tissue. Under these culture conditions, MSCs appear to be superior over ECCs for cartilage regeneration.

I. INTRODUCTION

Adult bone marrow-derived MSCs cultured as pellets [1] and in scaffolds [2] in chondrogenic media produce a cartilaginous extracellular matrix (ECM) similar to that of native articular cartilage tissue. The contraction of the constructs associated with chondrogenesis was attributed to the expression of α-smooth muscle actin (α-SMA) by the MSCs [2]. Furthermore, the mechanism of contraction has been termed “embryonic condensation,” the process of cell aggregation and compaction to increase cell-cell contacts necessary for chondrogenesis [3].

This study compares the potential of pluripotent embryonic carcinoma cells (ECCs) and MSCs for cartilage histogenesis in collagen scaffolds in vitro, and investigates the associations among contraction, α-SMA expression, and chondrogenesis. The results relate to the judicious selection of a cell source for cartilage tissue engineering, and shed light on the reasons for differences in embryonic/fetal and adult wound healing.

II. MATERIALS AND METHODS

Immortalized P19 mouse embryonic carcinoma cells (ATCC) and passage 3 adult porcine MSCs were used in the following studies:

A. Monolayer

Cells were cultured in chamber slides (5x10^4 cells per well) and cultured in complete medium (DMEM-LG, 10% FBS, 1% P/S) for 48 h. Monolayers were then stained using standard immunohistochemical techniques for α-SMA.

B. Pellets

Cells were added to 15mL conical tubes (2x10^5 cells per tube) and centrifuged to form pellets. Two pellets were washed in PBS and frozen as controls. The rest of the pellets were cultured in chondrogenic media [3] for various times. DNA and GAG content (n=2) were determined after proteinase K digestion. Samples for histology (n=6) were fixed and embedded in paraffin. Sections were stained with Safranin-O for GAG. Standard immunohistochemical methods were used to stain for α-SMA and type II collagen.

C. Scaffolds

Disks (8mm diameter, 2mm thick) of porous (120μm) type I/III and type II collagen scaffolds were fabricated by freeze-drying the respective porcine-derived collagen slurries (Geistlich Biomaterials). The scaffolds were sterilized and cross-linked by dehydrothermal treatment before additional cross-linking using carbodiimide. Scaffolds were seeded with 2x10^6 ECCs or MSCs (n=8), and cultured for 14 days in chondrogenic medium with non-cell-seeded scaffolds as controls. Scaffold diameters were measured every 2-3 days at each medium change. Biochemical assays and immunohistochemical evaluation were performed on the scaffolds after 14 days.

III. RESULTS

A. Monolayer

In monolayer culture, MSCs were larger and displayed prominent filament bundles as compared to ECCs (Fig. 1). Virtually all the MSCs and ECCs stained for α-SMA, but the epitope appeared to be more uniformly distributed in the cytoplasm of the MSCs.

B. Pellets

MSCs formed hard cartilaginous pellets at the end of 14 days whereas the ECCs formed a loose aggregate of cells that

![Fig. 1. α-SMA stain in (A) pig MSCs and (B) mouse ECCs (n=2).](image-url)
did not exhibit cartilage-like tissue formation (Fig. 2). The MSC pellets displayed tissue rich in sulfated GAG and type II collagen whereas ECC pellets did not.

The DNA content of ECC-pellet increased from 0.53 ± 0.03 µg to 3.7 ± 0.2 µg (n=2) whereas that of MSCs had no significant change (Fig. 3). The GAG content of MSCs increased from 0 to 14.5 ± 1.6 µg (n=2) whereas that of ECCs only increased from 5.3 ± 0.93 to 13.7 ± 2.2 (Fig. 3), indicating that MSCs formed GAG-rich tissue characteristic of articular cartilage.

Fig. 4 shows ECC pellets at different times. Although the pellets displayed a high cell density, they exhibited little ECM formation. The safranin-O stain shows little to no sulfated GAG in the pellets (Fig. 4F-J). The pellets stained strongly for α-SMA until day 14 and then ceased. None of the ECC pellets stained for Type II collagen (Fig. 4P-T).

**C. Scaffolds**

MSC-seeded scaffolds contracted to 60% of their original diameter over 14 days whereas ECC-seeded scaffolds did not show significant contraction. ANOVA testing did not show a significant difference in contraction data between types I and II collagen scaffolds. At the end of 14 days, MSC constructs produced GAG-rich ECM and type II collagen whereas ECC constructs did not (Fig. 5).

DNA content of ECCs (on type I scaffolds) increased from 6.0 ± 0.6 µg to 15.6 ± 0.5 µg whereas that of MSCs (on type I scaffolds) stayed constant (Fig. 6). GAG content of MSCs (on type I scaffolds) increased from 9.0 ± 1.0 µg to 300 ± 80 µg whereas that of ECCs (on type I scaffolds) increased from 10 ± 1 µg to 38 ± 3 µg. Similar trends in DNA and GAG contents were observed in type I and type II collagen scaffolds. ANOVA testing shows a significant increase in GAG:DNA ratio for MSCs than ECCs (Fig. 6).

**IV. DISCUSSION/CONCLUSIONS**

ECCs in pellets and on scaffolds did not form cartilaginous ECM under conditions in which MSCs did. MSCs contracted to form pellets while ECCs did not. Prior work demonstrated that it is the α-SMA-enabled contraction of MSCs that causes the pellet to form [1]. Formation of the pellet and its contraction causes “condensation” of cells required for chondrogenesis. The α-SMA-expressing ECCs may not have contracted due to the absence of actin unit polymerization or the absence of myosin molecules. These observations may explain the absence of a contractile scar in fetal wound healing. Under these culture conditions, MSCs appear to be superior over ECCs for cartilage regeneration.

**REFERENCES**

