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# Clonal analysis of synovial fluid stem cells to characterize and identify stable mesenchymal stromal cell/mesenchymal progenitor cell phenotypes in a porcine model: a cell source with enhanced commitment to the chondrogenic lineage

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**Abstract**

*Background aims.* Previous studies have demonstrated that porcine synovial membrane stem cells can adhere to a cartilage defect *in vivo* through the use of a tissue-engineered construct approach. To optimize this model, we wanted to compare effectiveness of tissue sources to determine whether porcine synovial fluid, synovial membrane, bone marrow and skin sources replicate our understanding of synovial fluid mesenchymal stromal cells or mesenchymal progenitor cells from humans both at the population level and the single-cell level. Synovial fluid clones were subsequently isolated and characterized to identify cells with a highly characterized optimal phenotype. *Methods.* The chondrogenic, osteogenic and adipogenic potentials were assessed *in vitro* for skin, bone marrow, adipose, synovial fluid and synovial membrane–derived stem cells. Synovial fluid cells then underwent limiting dilution analysis to isolate single clonal populations. These clonal populations were assessed for proliferative and differentiation potential by use of standardized protocols. *Results.* Porcine-derived cells demonstrated the same relationship between cell sources as that demonstrated previously for humans, suggesting that the pig may be an ideal preclinical animal model. Synovial fluid cells demonstrated the highest chondrogenic potential that was further characterized, demonstrating the existence of a unique clonal phenotype with enhanced chondrogenic potential. *Conclusions.* Porcine stem cells demonstrate characteristics similar to those in human-derived mesenchymal stromal cells from the same sources. Synovial fluid–derived stem cells contain an inherent phenotype that may be optimal for cartilage repair. This must be more fully investigated for future use in the *in vivo* tissue-engineered construct approach in this physiologically relevant preclinical porcine model.

**Key Words:** *chondrogenesis, mesenchymal stromal cells, porcine, synovial fluid, synovium*

## Introduction

Articular cartilage damage is a common clinical problem that has garnered extensive research attention during the past decade, particularly though stem cell modalities. Chondrocytes, which are a distinct and unique cell type located within cartilage, exhibit a limited repair capacity, meaning that damaged cartilage will not spontaneously regenerate. Advanced degradation of joints routinely leads to replacement by artificial joints. Whereas these biomedical devices have shown efficacy in restoring function, they are plagued by a number of side effects, are not durable for extended periods of time and are not as effective as an actual joint. Cell-based therapies are now being examined as a means to regenerate cartilage. Advances in tissue engineering, led by research into cell biology and biomaterials, are enabling regenerative medicine to emerge as a potential alternative to artificial joints. Cell therapies may provide a novel therapeutic approach for controlling progression of diseases that alter chondrocytic function as well as to promote cellular regeneration. Autologous chondrocyte implantation is a fairly recent cell therapy that involves the isolation chondrocytes from a cartilage biopsy taken from a healthy and minor load-bearing area of the afflicted

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joint surface (1). This procedure has undergone clinical trials demonstrating some long-term efficacy (2). However, in some patients, the repair tissue is a fibrocartilage (3) rather than hyaline-like, which may be attributable to the fact that culture expansion of chondrocytes may lead to dedifferentiation or an unstable phenotype and inevitably a loss of chondrogenic potential *in vivo* (4). In addition to this limitation, the chondrocyte biopsy itself may result in further joint damage and so the need has arisen for a new source of cells that may be used therapeutically, ultimately in a minimally invasive fashion and with a stable phenotype. Mesenchymal stromal cells (MSCs) are promising because they are highly proliferative, undifferentiated cells with the potential to expand extensively and differentiate into several lineages (5). Currently, the outcomes of cell therapies are inadequate; one of the primary complications is caused by difficulties in maintaining a stable chondrocytic phenotype. Autologous cell therapies with the use of highly characterized clonal MSCs or mesenchymal progenitor cells (MPCs) may be a viable alternative because cells can be selected with optimized properties for the specific application of interest. It is known that synovial fluid (SF) MSCs/MPCs from injured or diseased joints may not be ideal because they can be compromised by an inflammatory microenvironment (6,7). It is well documented that human synovium contains an inherent MSC/MPC population with an enhanced propensity for chondrogenesis (8). Recently, we demonstrated that porcine synovial cells exhibit enhanced chondrogenic potential and can repair cartilage *in vivo*, (9) similar to that demonstrated in human synovium-derived stem cells (8). We have also demonstrated this previously in a porcine model (10) as well as with human MSCs (11). However, the cell populations used in these studies probably were a mixed heterogeneous population because they were derived from multiple clones, and, as such, phenotypic stability may be questionable because of the presence of heterogeneity between subsets during culture expansion (12). SF contains an inherent stem cell population (13) that can be accessed with minimally invasive methods and requires less processing than synovium, reducing the risk of contamination. In particular, the use of collagenase has been linked to potential bovine spongiform encephalopathy contamination. Because of the ease of isolation and less need for extensive processing, SF-derived cells may be an ideal candidate cell source for clinical applications; however, further examination is needed. Our initial studies indicate that SF may be able to regenerate cartilage more efficiently than other tissue types. Because of the heterogeneity that is present in stem cell populations, examining the single clonal population may also be a more effective method of repairing cartilage by increasing the consistency and predictability of these clonal cells. Therefore, the objectives of the current studies were to both confirm that porcine SF is an effective cell source for chondrogenesis and to establish the proliferative and differentiation characteristics of individual clonal populations, which could ultimately be used to select highly characterized cells with a defined and optimal phenotype. This would provide an opportunity to optimize the tissue-engineered construct (TEC) approach in a porcine model before clinical investigation.

## Methods

### *Collection and isolation of cells from porcine knees*

The animal protocols were approved by the institutional ethics committee at the University of Calgary Health Sciences Centre. SF was obtained from the hind legs of three female juvenile Yorkshire pigs between 3–4 months old and weighing 25–30 kg (Table I). After euthanasia, a medial parapatellar incision was made and the SF was aspirated with the use of a 23-gauge needle before opening the knee capsule. After ensuring complete aspiration of SF, 10 mL of sterile pyrogen-free phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA, USA) was injected to expand the knee joint space, and this fluid was then re-aspirated. The cell isolation protocol for synovial membranes (SM) was identical to that described previously (10). Briefly, SM specimens were obtained aseptically from the knee of the same pig after collecting SF, and the tissue was then minced meticulously. The minced tissue was digested with 0.2% collagenase (Worthington Biochemical Corp, Lakewood, NJ, USA). After removal of full-thickness skin, adipose tissue cells were obtained from the subcutaneous tissues, and the same procedure was followed as that for the cell isolation of SM. Bone marrow (BM) was obtained from the femurs of each pig by aspiration. Freshly isolated cells were diluted 1:4 with PBS and obtained by centrifugation. All cells were then resuspended in basic culture growth media containing high-glucose Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% antibiotic/antimycotic (Invitrogen Canada Inc) and were then plated in T-25 cell culture flasks (BD Bioscience, San Jose, CA, USA). Non-adherent cells were removed by changing the media. Cells were then

Table I. Characteristics of porcine subjects.

| Subject | Age, months | Sex | Weight, kg | Passage |
|---|---|---|---|---|
| A | 3 | Female | 26.2 | 3 |
| B | 3 | Female | 29.3 | 3 |
| C | 4 | Female | 27.8 | 3 |