In vitro cartilage formation via co-culture of primary articular or non-articular chondrocytes with bone marrow- or adipose-derived mesenchymal stromal cells

Adetola Adesida1,2, Andrea Barbero2, Chitrangada Achaya1, Jens Riese1 and Ivan Martin2
1CellCoTec B.V., Bilthoven, The Netherlands 2University Hospital Basel, Basel, Switzerland
adetola.adesida@cellcotec.com

Introduction: the generation of target tissue through co-culture of tissue-specific cells with multipotent stem cells is increasingly recognized as a potent regenerative medicine strategy (1, 2). Cartilage formation via co-culture of chondrocytes and bone marrow derived mesenchymal stem cells (BMSCs) has been demonstrated as a strategy that simultaneously solves major limitations of autologous chondrocyte and multipotent stem cell use in cell-based tissue engineering protocols to repair cartilage defects, namely low chondrocyte yield, dedifferentiation of expanded chondrocytes, and compromised chondrogenic capacity associated with passaged stem cells. However, bovine chondrocytes were used therein (3). Thus, with the ultimate goal to identify clinically relevant cell sources, here we investigated cartilage formation via co-culture of primary human chondrocytes (PC) from articular and non-articular cartilages with human mesenchymal stem cells from bone marrow or adipose tissues.

Materials and Methods: Biopsies were obtained following approval of the local ethical committee and informed consent. Articular cartilage was harvested from the femoral condyles and tibial plateau of 7 human cadaver knees within 24–48 hr of death (all male; age 26–84 years; mean 59) and 3 donors undergoing total knee arthroplasty; 1 male (68) and 2 females (age 56 and 63). Nasal cartilage was from 2 donors; male (37) and female (36) undergoing plastic and reconstructive surgery. Primary human chondrocytes were obtained after cartilage digestion with 0.15% type II collagenase at 37°C for 22 h, in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum (FBS). Freshly isolated chondrocytes were suspended in DMEM with 10% FBS. Chondrocytes were plated for 24-48 hr before use. Bone marrow aspirates were obtained from the iliac crest of 5 donors: 1 female (35) and 4 males (mean age; 47) undergoing routine orthopaedic procedures. Nucleated cells in the aspirates were isolated as described before (4), followed by expansion in α-minimal essential medium (α-MEM) supplemented with 10% FBS and basic fibroblast growth factor (FGF-2) at 5 ng/ml. Total population doublings was 13-17. Subcutaneous adipose tissue in the form of excised infrapatellar fat pad from 2 male cadavers (62 and 67), and lipoaspirates from 3 abdominal-plasty donors (1 male and 2 females, mean age of 43; from the thigh of 2 females (39 and 35) were obtained. Adipose tissue were digested by incubation with 0.15% type II collagenase at 37°C for 1 h in phosphate buffered saline (PBS). Nucleated cells from the resulting stromal vascular fraction were plated and cultured in α-MEM with FGF-2 as described previously. Total cell population doublings was 6-8. Cells were mixed at a variety of PC:MSC ratio including 5/95, 10/90 and 25/75 depending on isolated or available cell numbers. Mixed cells were centrifuged to form spherical pellets in a defined serum free medium as previously described for in vitro chondrogenic differentiation (5, 6). Dexamethasone was excluded in co-cultures involving ADSCs (7). Total cell number per pellet was 4 x10^5. For control groups, single cell type pellets, i.e. 100% PC and MSCs, were formed. Pellets were cultured for 3 weeks in a humidified incubator at 37°C and 5%CO2. Thereafter, pellets were processed biochemically for glycosaminoglycans (GAG) and DNA content, and histologically for Safranin-O staining of sulphated GAG and type II collagen deposition. Analysis was performed on a minimum of two independent whole pellets.

Results: Five of seven co-cultures of primary human articular chondrocytes (AC) and hBMSCs, and three of seven co-cultures of AC and adipose-derived mesenchymal stromal cells (ADSC) formed cartilaginous tissues as confirmed by positive Safranin-O staining and type II collagen labeling. In similar co-cultures, regardless of the MSC source, but with primary nasal chondrocytes (NC), neo-cartilage formation was confirmed. In all co-cultured cell pellets where neo-cartilage formation was evident, increased GAG production was also observed relative to control pellets from 100% chondrocytes or 100% MSCs. However, neo-cartilage formation was independent of the intrinsic chondrogenic capacity (as judged by GAG/DNA content) of the primary chondrocytes or MSCs alone. Representative data where in neo-cartilage formation was evident post co-culture of AC (male cadaver; 53 year old) or NC (male, 37 year old) with hBMSCs (male, 53 year old) is shown in Fig.1. Relative to control pellets, the chondrogenic capacity (as judged by GAG/DNA content or induced GAG) of pellets from 10% AC or 10% NC with 90% hBMSCs co-cultures, increased by 1.3-fold and 1.5-fold, respectively (Fig.1 A and B). In addition, the pellets derived from these co-cultures stained positively for sulphated GAG by Safranin-O stain (Fig.1C) and type II collagen protein (Fig.1D).

Conclusion: We demonstrate that co-culture of primary chondrocytes from articular or nasal cartilage with bone marrow or adipose derived MSCs is a strategy that provides chondro-inductive signals for neo-cartilage formation. The mechanism of chondro-induction or molecular characteristics involved is currently unknown. Nonetheless, it is plausible that cell-cell contact communication via molecular compatible gap junctions exist between primary chondrocytes and MSCs (8). Together with recent reports on the plasticity of NC (9) and of ADSC, our data open the perspective to induce cartilage tissue regeneration by co-delivery of cell types, which can be harvested under minimally invasive conditions and with minimal donor site morbidity.

Figure 1. Representative GAG/DNA content, Safranin-O staining and type II collagen immunohistochemistry of pellets derived from co-cultures of primary articular (AC) or nasal (NC) chondrocytes with human hBMSCs.

References
2. Richardson SM et al. Stem Cells 2006;24(3):707-16.