INTRODUCTION:
Adult articular cartilage exhibits a poor regenerative capacity following injury, and this inability to regenerate is a major factor contributing to the development of osteoarthritis and joint degenerative disease following cartilage injuries. Cell-based therapies such as autologous chondrocyte implantation (ACI) are of interest to enhance the natural regenerative capacity, replace acutely damaged cartilage and inhibit progression of disease. Expansion of chondrocytes in vitro is required to generate adequate populations for ACI. However, cell passaging during standard methods causes “dedifferentiation” into fibroblastic cells [1]. To circumvent chondrocyte dedifferentiation during population expansion, we developed a novel culture technique that facilitates continuous growth of cells while limiting effects of contact inhibition and reducing the need for passaging. Here, we investigated the effects of the surface expansion culture system on chondrocyte dedifferentiation compared to standard culture.

METHODS:
Continuous Expansion Culture. Bovine chondrocytes were isolated from dissected articular cartilage from the femoropatellar groove of skeletally mature cows using established methods [2]. 10,000 chondrocytes/cm² were seeded and subcultured in chondrocyte growth medium. Continuous expansion (CE) cultures were performed on high extension silicone rubber (HESR) dishes which were expanded from dissected articular cartilage from the femoropatellar groove of 12-month-old animals. Pellets were then fixed and stained with alcian blue to observe chondrocyte outgrowth. Pellets were then transferred to a six-well plate and incubated for an additional six days to observe chondrocyte outgrowth. Pellets were then fixed and prepared for cryosectioning. Sections were stained with 1:500 smooth muscle antibody (SMA; 1:500, Abcam) and secondary TRITC conjugated goat anti-mouse IgG (Sigma).

Real-Time qPCR. Following Trizol (Invitrogen) RNA extraction, 500 ng of total RNA was subject to cDNA synthesis using a qScript cDNA kit (Quanta Biosciences, Gaithersburg, MD). 1 µl of each cDNA sample was loaded per reaction (in duplicate) using PerfeCTa SYBR Green FastMix (Quanta Biosciences). PCR was performed using the ABI 7900 HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA). With GAPDH as endogenous control, the average fold change in gene expression at the end of each generation of CE culture was compared to the final passage of each generation of SD culture was calculated.

Western Blot. Chondrocytes from each generation were lysed and subjected to SDS-PAGE and transferred to nitrocellulose membranes. Blocked membranes were probed with antibodies against smooth muscle actin (α-SMA; 1:500, Abcam) and α-tubulin (1:2000, Abcam), followed by anti-mouse HRP-conjugated IgG (1:5000, Cell Signalling).

RESULTS:
Continuous expansion culture of primary bovine chondrocytes yielded a somewhat smaller cell population with a more chondrocyte-like morphology and higher RNA-level expression of the chondrogenic markers collagen type II, aggrecan and COMP versus standard culture. Expression of collagen type I was significantly reduced and Western blot analysis revealed suppression of α-SMA expression in generations G1 and G2. Pellet cultures from continuous expansion chondrocytes secreted more sulphated glycosaminoglycans and generated more collagen type II protein compared to pellets from standard culture.

DISCUSSION:
Chondrocytes grown in CE culture were passaged 4 times less than SD culture, and perhaps as a result retained a more rounded morphology and cartilage-specific gene expression, and reduced fibrocytic gene and protein expression. Chondrocytes from CE culture were superior to those from SD culture with respect to their ability to redifferentiate toward a terminal chondrocyte phenotype in pellet culture. Both SD and CE pellets initially were able to produce GAG and collagen type II, however only CE pellets maintained this production throughout three generations. Taken together, these results indicate that continuous expansion culture produces cells which are markedly superior to those obtained from standard culture, with respect to efficiently redifferentiating and generating de novo cartilage-like tissue.

SIGNIFICANCE:
Chondrocytes grown in continuous expansion culture may be a superior source for cell-based therapies.

REFERENCES: