MesenCult™-ACF Chondrogenic Differentiation Medium – A Robust Animal Component-Free Chondrogenic Stimulatory Medium for the Efficient Differentiation of Human Mesenchymal Progenitor Cells

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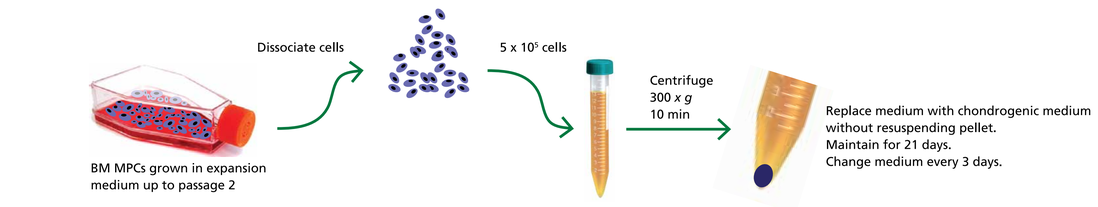
Introduction

Human bone marrow (BM)-derived mesenchymal progenitor cells (MPCs) expanded in serum-containing or serum-free media are routinely used as a source of cells for chondrogenic differentiation *in vitro*. Efficient differentiation of MPCs is challenging, however, due to the inconsistency of sera used in homemade and commercial media. To avoid the use of serum, we have developed MesenCult™-ACF Chondrogenic Medium (ACF-Ch), a defined and animal component-free (ACF) chondrogenic medium that supports consistent differentiation of culture-expanded MPCs. Human mononuclear cells (MNCs) were isolated from fresh BM and expanded in either commercial Medium A, commercial Medium B, MesenCult™ (serum-containing), MesenCult™-XF (xeno-free), or MesenCult™-ACF media. Cells were then harvested and maintained for 21 days in either ACF-Ch or one of three commercial chondrogenic differentiation media (Com-Ch1, Com-Ch2, or Com-Ch3) with complete media changes performed every 3 days. MPCs expanded in MesenCult™, MesenCult™-XF, or MesenCult™-ACF and differentiated in ACF-Ch formed denser cartilage as demonstrated by positive Alcian Blue (AB) staining and contained more evenly distributed isogenous groups with minimal hypertrophic areas, as observed by Nuclear Fast Red (FR) staining, than MPCs differentiated in Com-Ch1, Com-Ch2, or Com-Ch3. The expression levels of chondrocyte-specific transcripts in MPCs differentiated in either ACF-Ch or Com-Ch1 (day 9) or Com-Ch2 (day 21) were normalized to *Tbp* expression and it was found that ACF-Ch consistently induced earlier and stronger expression of key transcripts compared to commercial media. Whether characterizing culture-expanded MPCs or differentiating MPCs for research, use of MesenCult™-ACF Chondrogenic Differentiation Medium consistently supports robust chondrogenic differentiation of MPCs.

Materials and Methods

Expansion and Differentiation

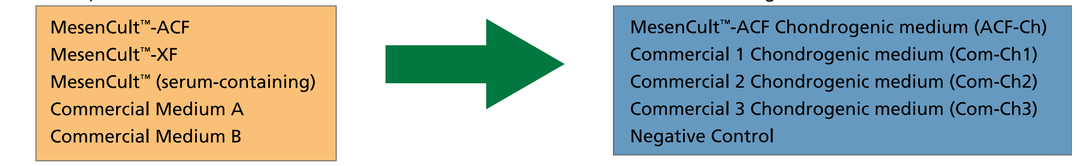
FIGURE 1: General protocol for the isolation, expansion, and chondrogenic differentiation of BM MPCs



Fresh BM MNCs were isolated and grown in expansion media (Fig. 2) for up to 2 passages. Cultures were then dissociated, counted, and 3D culture pellets containing 5 x 10^5 cells were setup in 15 mL tubes. Differentiation cultures were maintained for up to 21 days with media changes every 3 days.

Detailed protocols for the isolation, expansion and maintenance of human BM MPCs can be found in the STEMCELL™ Technologies Inc. Technical Manual: Culture of Human Mesenchymal Stem Cells Using MesenCult™-XF Medium (Document #29184).

FIGURE 2: Media used in the isolation, expansion, and differentiation of BM MPCs



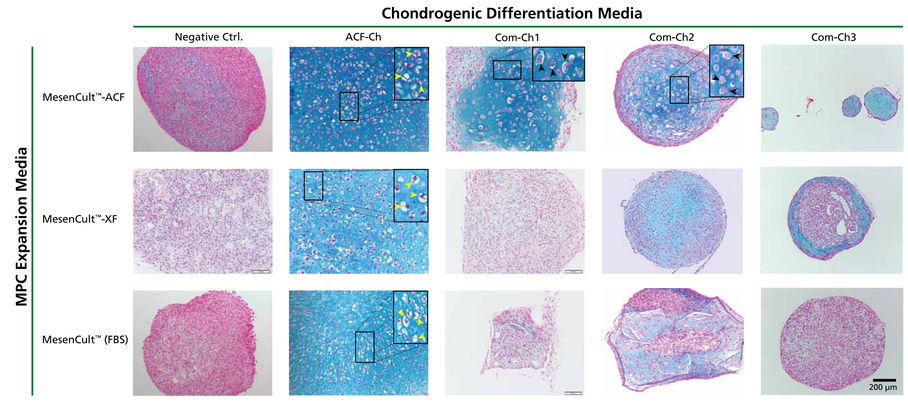
A) MNCs were isolated from fresh BM and MPCs expanded up to passage 2 in each medium. **B)** Differentiation cultures were setup in each of the chondrogenic differentiation media. A negative control devoid of chondrogenic agents was also used.

Analysis

Cell culture pellets were either stained with AB and FR at day 21 or processed at day 9 and 21 for qPCR analysis of specific chondrogenic transcripts. Histological sections of differentiated cultures were prepared by the University of British Columbia Hospital Histochemistry Laboratory. Culture sections were stained with AB to show the cartilage matrix (glycosaminoglycan) and counter-stained with FR to show the 3D cell arrangement. qPCR analysis was performed using ABI 2-step fast reaction technology and TaqMan Probe chemistry with algorithm-validated primer/probe sets for the human chondrogenic transcripts *Acan*, *Col2a*, *Sox9*, *Col10a*, and *Mmp13*, which were obtained from Integrated DNA Technologies (IDT). Chondrogenic gene expression was normalized to internal *Tbp*.

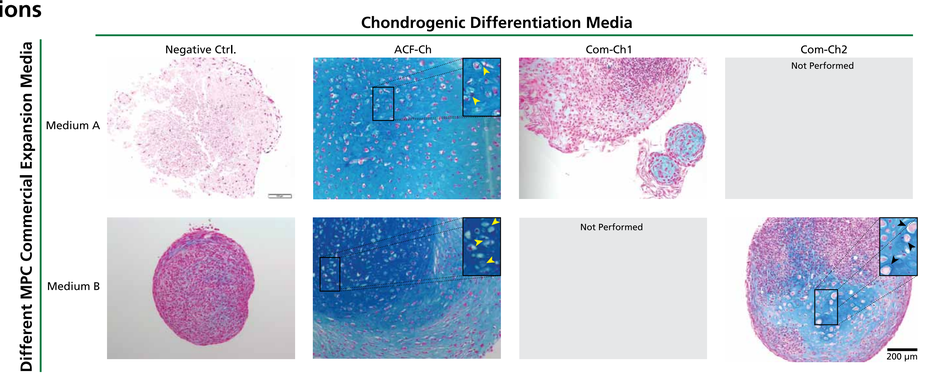
Results

FIGURE 3: ACF-Ch induced stronger and more uniform chondrogenic differentiation of BM MPCs expanded in MesenCult™-ACF, -XF, or FBS than Com-Ch1, Com-Ch2, or Com-Ch3



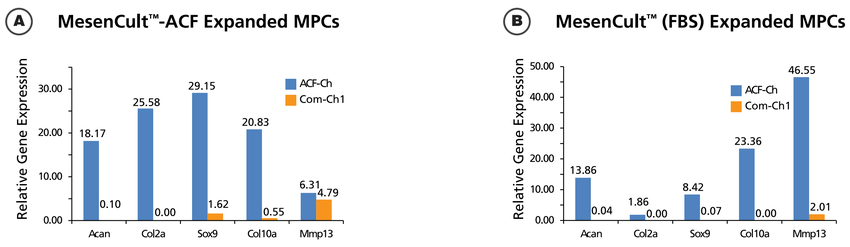
Culture of expanded BM MPCs in ACF-Ch induced stronger and more uniform chondrogenic differentiation after 21 days than Com-Ch1, Com-Ch2, or Com-Ch3. Cultures differentiated in ACF-Ch displayed an abundance of isogenous groups (insets, yellow arrowheads) with few hypertrophic chondrocytes. Cultures expanded in MesenCult™-ACF and differentiated in Com-Ch1, Com-Ch2, or Com-Ch 3 were only partially differentiated and displayed an increased number of hypertrophic chondrocytes (insets, black arrowheads). Cultures expanded in either MesenCult™-XF or MesenCult™ (FBS) and differentiated in Com-Ch1, Com-Ch2, or Com-Ch3 showed little or no AB staining, suggesting that these cells differentiated poorly and largely resembled the undifferentiated negative control cells.

FIGURE 4: ACF-Ch induced strong chondrogenic differentiation of BM MPCs expanded in different commercial formulations



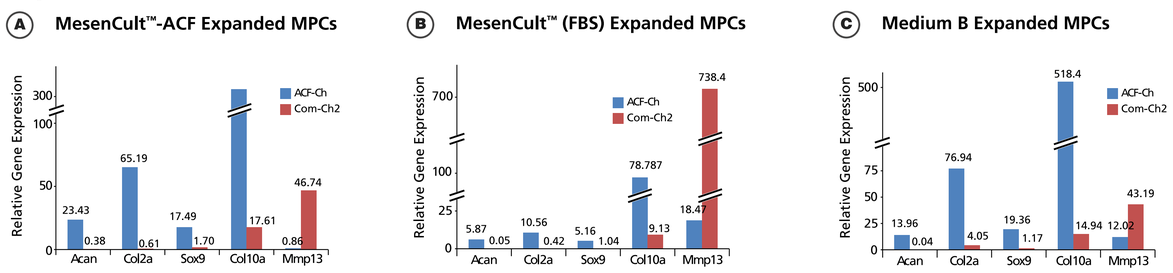
BM MPCs were expanded in Medium A and differentiated with Com-Ch1 medium or expanded in Medium B and differentiated with Com-Ch2. Medium A and Medium B-expanded cells were also differentiated with ACF-Ch and the negative control media. Cultures differentiated with ACF-Ch showed robust chondrogenic differentiation as demonstrated by AB staining and contained numerous isogenous groups (insets, yellow arrowhead). Cultures expanded in Medium A and differentiated with Com-Ch1 showed minimal and localized chondrocyte differentiation. Cultures expanded in Medium B and differentiated in Com-Ch2 showed localized chondrocyte differentiation with numerous hypertrophic chondrocytes (insets, black arrowheads). The negative control cultures did not differentiate.

FIGURE 5: ACF-Ch induced earlier and stronger expression of chondrogenic transcripts than Com-Ch1 in Day 9 differentiation cultures



A) BM MPCs were expanded in MesenCult™-ACF and differentiated in ACF-Ch or Com-Ch1 media for 9 days. ACF-Ch differentiation led to a substantial upregulation of the cartilage matrix transcripts *Acan* and *Col2a*, the transcription factor *Sox9*, and the maturation gene *Col10a* compared to Com-Ch1. Differentiation with Com-Ch1 led to little or no expression of these transcripts. Expression of the terminally-differentiated hypertrophic transcript *Mmp13*, however, was highest in Com-Ch1 than in ACF-Ch. **B)** BM MPCs expanded in MesenCult™ (FBS) and differentiated in ACF-Ch showed a substantial increase in all chondrogenic transcripts compared to Com-Ch1. Numbers above bars are the relative fold gene expression normalized to *Tbp* expression (n = 1).

FIGURE 6: ACF-Ch induced stronger and sustained expression of chondrogenic transcripts than Com-Ch2 in Day 21 differentiation cultures



A) BM MPCs were expanded in MesenCult™-ACF and differentiated with ACF-Ch or Com-Ch2 for 21 days. ACF-Ch differentiation led to a substantial upregulation of the cartilage matrix transcripts *Acan* and *Col2a*, the transcription factor *Sox9*, and the maturation gene *Col10a* compared to Com-Ch2. Differentiation with Com2 led to little transcript expression. Expression of the terminally-differentiated hypertrophic transcript *Mmp13* however, was highest and >50-fold higher compared to ACF-Ch. **B)** BM MPCs expanded in MesenCult™ (FBS) and differentiated in ACF-Ch showed a substantial increase in all chondrogenic transcripts compared to Com-Ch2. *Mmp13* expression was 40-fold higher compared to ACF-Ch. **C)** BM MPCs expanded in Medium B and differentiated with ACF-Ch also showed increased expression of all chondrogenic transcripts when compared to Com-Ch2. *Mmp13* expression was 3.6-fold higher in Com-Ch2. Number above bars represents the relative gene expression normalized to *Tbp* expression (n = 1).

Conclusions

MesenCult™-ACF Chondrogenic Differentiation Medium Provides:

Efficient chondrogenic differentiation of bone marrow-derived mesenchymal progenitor cells (BM MPCs)

- Consistent and robust chondrogenic differentiation of BM MPCs expanded in various media.

Consistent commitment and strong chondrogenic differentiation of BM MPCs

- Large chondrogenic culture pellet formation containing a substantial number of isogenous groups, suggesting the proliferation of differentiating chondrocyte progenitors.
- Less hypertrophic chondrocytes, suggesting the maintenance of chondrogenic activity throughout the culturing period.

Increased upregulation of chondrogenic-specific transcripts

- Strong induction of expression of chondrogenic transcripts *Acan*, *Col2a*, *Sox9*, *Col10a*, and *Mmp13* at days 9 and 21 of differentiation.