Enhanced \textit{in vitro} Cartilage Formation by Co-Culture of Human Primary Chondrocytes and Mesenchymal Stromal Cells

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\section*{Introduction}
Co-culture of mesenchymal stromal cells (MSC) with chondrocytes (Ch) has been reported to improve cartilaginous matrix accumulation\textsuperscript{1} (phenomenon here named chondro-induction, CI). In this study, we investigate the type(s) of communication between the two cell types responsible for CI.

\section*{Materials and Methods}
MSC were isolated from human bone marrow (BMSC, N=13) and expanded \textit{in vitro} for two weeks. Human primary articular Ch (pAC, N=22) isolated from knee cartilage tissues were used without expansion. The two cell types were cultured in pellets [alone (pure pellets) or after being mixed (co-culture pellet) at a pAC: MSC ratio of 25\%:75\%] for 3 weeks in medium containing TGF\textbeta\textsubscript{1}. Selected pellets were generated combining: (i) human pAC with human dermal fibroblasts (i.e. cells unable to chondro-differentiate), (ii) human BMSC with bovine pAC, and (iii) BMSC from HLA-A2+ with pAC from HLA-A2- donors. BMSC and pAC were also cultured in transwells, with the two cell types physically separated.

Pellets were assessed biochemically [to quantify CI as a ratio $\frac{\text{GAG}_{\text{measured}}}{\text{GAG}_{\text{expected}}}$ ($\text{GAG}_{\text{expected}} = 75\% \text{GAG}_{\text{pure MSC}}+25\% \text{GAG}_{\text{pure pAC}}$)], by RT-PCR using human and bovine specific primers and probes for types I and II collagens (to assess gene expression changes in the two cell populations), and cytofluorimetrically (to quantify variation in cell numbers). Tissues formed in the inserts of the transwells were assessed histologically and biochemically.

\section*{Results}
CI was higher when pAC were co-cultured with BMSC (1.6±0.1) than with dermal fibroblasts (1.3±0.1). RT-PCR of pellets generated by bovine pAC and human BMSC showed an increase in the expression of human (and not bovine) type II collagen following co-culture (Fig.1).

\begin{figure}[h]
\includegraphics[width=0.5\textwidth]{fig1}
\caption{RT-PCR with specific primers and probes FACS quantification with antibodies specific for HLA-A2 indicated that: pAC increase (4.2-fold) in the co-culture pellets while remaining constant in pure pellets, BMSC decrease in the co-culture and pure pellets to a similar extent (5.0-fold) (Fig 2).}
\end{figure}

\section*{Discussion and Conclusions}
Co-culture of freshly isolated Ch with expanded MSC provide chondro-inductive signals for neo-cartilage formation. While soluble factors alone cannot account for this process, cell-cell contacts play key roles: pAC stimulate BMSC for higher type II collagen expression and, in turn, BMSC stimulate pAC to proliferate. \textit{In vivo} studies are required to assess the clinical relevance of our findings in the context of cartilage repair.

\section*{References}

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\section*{Disclosures}
Authors declare no conflict of interest.