Effect of Human Fibroblasts Over the Osteoblastic Activity of Human Osteoblastic-like Cells: Role for Cell-Cell Interactions

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Statement of Purpose: Most of the processes in human body require the coordinated action of more than one type of cells. The outcome is achieved through communication paths which are dependent on the cell-cell direct contact and/or on an indirect effect resulting from the production of soluble biochemical factors.

The aim of this work was to develop and characterize an *in vitro* co-culture model of osteoblasts and fibroblasts by assessing the effect of the fibroblasts in the osteoblasts proliferation rate and in their ability to produce alkaline phosphatase (ALP). Moreover, it was possible to show that Gap-junctional communication (GjC) is occurring between the two cell types with significant effect in osteoblastic activity.

Materials and Methods: An *in vitro* co-culture model of a human osteoblast cell line (SaOs-2) and a human fibroblast cell line (MRC-5), was used. SaOs-2 were cultured alone, in co-culture with MRC-5 and in the presence of MRC-5 conditioned medium for 2, 5, 7 and 9 days. In order to find out about a possible contribution of GjC in the observed cellular reaction, a GjC inhibitor (18-α-glycyrrethinic acid) was also used in the co-cultures. This inhibitor was added to the cultures each time the culture medium was replaced (every 2 days).

For dsDNA and alkaline phosphatase (ALP) activity quantification after each time point, cells were lysed by osmotic shock followed by incubation at 37ºC and freezing at -80ºC. The number of cells (used to normalize ALP) was obtained by fluorescence detection of the Carboxyfluorescein Diacetate Succinimidyl Ester (CFDA-SE) fluorescent probe.

Morphological analysis of the single cultures and of the co-cultures was performed by light microscopy. Osteoblasts were specifically stained for ALP and counterstained with hematoxylin which also allowed for a clear distinction between osteoblasts and fibroblasts in co-culture. Fluorescence microscopy was used to confirm the hypothesized GjC between SaOs-2 (marked with calcein-AM) and MRC-5 through the transference of gap junction (Gj) permeable calcein.

Results and Discussion: No morphological changes were detected, on both osteoblastic and fibroblastic cells, among the different culture conditions. The transference of calcein between SaOs-2 and MRC-5 also confirmed the GjC involving these two types of cells.

The dsDNA quantification showed that cell proliferation is delayed, for early times of culture, when SaOs-2 and MRC5 are co-cultured. However, the number of cells in co-culture reaches the addition of the number of cells in the isolated cultures from day 7 on. The proliferation rate of the osteoblasts was not affected by the fibroblast conditioned medium. In addition the comparison of the proliferation rate between co-cultures in the presence and absence of Gj inhibitor cultures did not show significant differences. The cell quantification results showed that osteoblast-fibroblast contact through GjC does not have a significant role in the proliferation of these cells under the studied conditions.

The effect of MRC-5 in the osteoblastic activity of SaOs-2 was detected by ALP quantification (figure 1). In fact, the presence of MRC-5 cells inhibited ALP production. This effect was only achieved by direct cell-cell contact, since no significant difference was noted in ALP production in SaOs-2 cells cultured with MRC-5 conditioned medium. Furthermore, the observed effect was proven to involve GjC because no significant difference was noted when comparing ALP production in isolated SaOs-2 cells and in SaOs-2 cells cultured with MRC-5 and GjC inhibitor.

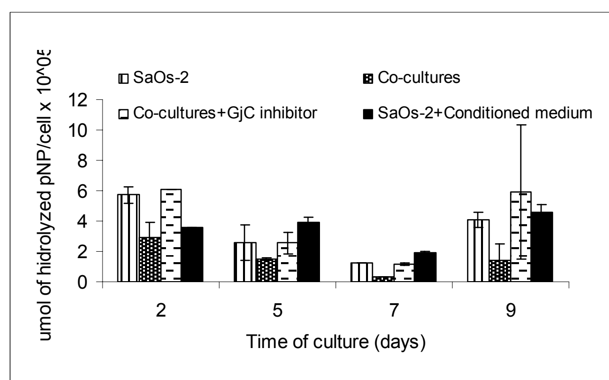

Figure 1. Amount of ALP production per osteoblast under different culture conditions after 2, 5, 7 and 9 days. Results are presented as mean ± standard deviation.

Conclusions: The developed co-culture model showed that SaOs-2 proliferation was not directly or indirectly affected by MRC-5 cells. In addition, MRC-5 inhibited SaOs-2 *in vitro* ALP production through direct cell-cell contact and in particular through gap junctional communication. The cellular interactions proven to occur, render this *in vitro* co-culture model as a useful tool for a better understanding and eventually controlling of the cellular response to *in vitro* cultured tissue engineering scaffolds.

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