Platelet Lysate Promotes Wound Closure of Human Keratinocytes Under Physiologic And Inflammatory Conditions
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Introduction
Platelet rich concentrates have been in clinical use in maxillofacial and orthopedic surgery for over a decade. They contain numerous growth factors and cytokines, many of which play pivotal roles during the inflammatory phase of wound healing. During wound healing, the inflammatory phase represents the triggering events for the regenerative process. While the role of platelet lysates on cell proliferation has been demonstrated and some basic research has been carried out about factors contained in platelet lysate, there is little or no information about the pathway/s induced by platelet lysate to the tissue leading to repair. The aim of this study was to study the effects of platelet lysate in physiological and inflammatory on wound closure of human keratinocytes (NCTC 2544).

Materials and Methods
Platelet lysates (PL) were prepared from human PRP ¹ at a concentration of 1 x 10⁷ platelets/µl which were resuspended in physiologic saline to eliminate all traces of plasma. Wound scratch assays were performed using confluent NCTC 2544 cells and incubated with serum-free MEM, MEM+ FBS, PL + MEM, PL + MEM+FBS, PL + MEM, and PL + MEM+FBS. Scratches were evaluated at different times post-scratching using the TScratch software². To evaluate the effects of PL on the expression of soluble factors, cells were stimulated with serum-free media, IL(1), PL, and IL(1) +PL and analysed for the expression of NGAL and IL-8 using western immunoblotting and Q-RT-PCR. Modified wound scratch assays were then performed whereby cells were first pre-stimulated with IL(1) and then subjected to the previously described scratch assay and evaluated at 0, 6 and 24 hours post-scratching. Cytoskeletal changes reflecting cell migration were evaluated by immunohist. analysis and Q-RT-PCR analysis.

Results
PL at a concentration of 5% enhanced wound closure rates of NCTC keratinocytes, in particular after 24 hours of scratching, when the wound had almost attained complete closure (Fig.1). These effects were also superior to those observed with 10% FBS alone (Fig.2). Under inflammatory conditions, PL5% also maintained the ability to induce enhanced migration at rates comparable to those seen with 10%FBS and statistically significant from those of the serum-free controls. Furthermore, expression of IL-8 and NGAL was up-regulated (Fig.3).

Discussion and Conclusions
Buffered platelet lysate enhanced wound closure in both physiologic and pathologic conditions. The upregulation of the expression of NGAL and IL-8 following stimulation indicate a pro-inflammatory effect of PL possibly involved in wound healing as well as a distinct antibacterial effect.

References