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PLATELET LYSATE FORMULATION BASED ON CHITOSAN FOR THE TREATMENT OF BUCCAL LESIONS: IN VITRO EVALUATION

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Platelets are specialized secretory cells that release, in response to activation, a large number of growth factors (GFs).
Platelet lysate (PL) and platelet rich plasma (PRP) are therefore proposed in the treatment of soft and hard-tissue surgical conditions and in the management of non-healing wounds.
Aim of the work was to develop formulations suitable to maintain PL in contact with injured tissues for a time suitable to treat lesions of oral cavity.
Chitosan was chosen as a basis for the vehicle due to its well known mucoadhesive and wound healing properties [2, 3]. In particular chitosan glutamate (CSG) and hydroxypropylcellulose (HPMC) high viscosity were used to optimize the rheological and mucoadhesive properties of the vehicle and to improve the resistance towards the removal effect of salivary flux. The vehicle was steam sterilized at 121℃ for 15 minutes. The formulation was based on 1:1 mixture of vehicle and PL obtaining a final concentration of CSG and HPMC of 3% w/w and 1% w/w respectively.
The concentration of platelet derived growth factor PDGF AB in the formulations was evaluated by means of ELISA test. The healing enhancement properties of the formulation (PL CGS) were evaluated on fibroblast cell line.
An in vitro wound healing test was used as a proof of concept of the wound healing properties. Fibroblasts were seeded in the 2 chambers (at 10^5 cells/cm^2) of an insert that are divided by a septum of 500 µm ± 50 µm. After 24 h, at confluence, the insert was removed displaying 2 areas of cell substrates divided from the prefixed gap. Cell substrates were put in contact with 200 µl of PL at 1/20 concentration and PL CGS diluted at the same PL concentration. At prefixed times (0, 24, 48, 72 h) microphotographs were taken to evaluate cell growth in the gap.
Figure 1 shows microphographs of the gaps in fibroblast substrates after the contact with: a) PL at 1/20 concentration, b) PL CGS at PL concentration of 1/20 after 24, 48 and 72 h of contact time. At 24 h fibroblasts started to invade the gap both in he substrate in contact with PL and in that in contact with formulation. At 48 h the invasion of the fibroblasts was complete and the gap could not be seen any more in both substrates. At 72 h the cells were subconfluent in both substrates.

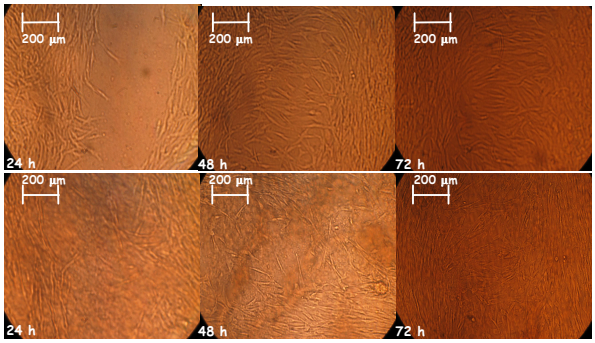

Fig. 1: microphotographs of gaps in fibroblast substrates after 24, 48 and 72 h of contact with: PL at 1/20 concentration (upper) and PL CGS at PL concentration of 1/20 (lower)

Concluding, the rheological and mucoadhesive properties of PL CGS formulation suggest that it possesses suitable properties to hinder removal action of salivary flux and to maintain a prolonged contact of PL with the damaged mucosa of the oral cavity. Chitosan vehicle is compatible with PDGF AB growth factor. The wound healing effect induced by PL CSG formulation is fast and analogous to that of PL. Platelet lysate loaded in chitosan vehicle has a positive effect on regeneration properties.

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