Aloe vera pectin: from extraction to matrices for regenerative medicine

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Statement of Purpose: Pectins, contained in the primary cell walls of plants, are a family of polysaccharides, rich in galacturonic acid partially methyl-esterified. Due to their biocompatibility, biodegradability and polyanionic nature, that allows for formation of hydrogel networks, these polymers have received increasing research interest for surface modification of medical devices and as carrier for drug delivery [1]. Pectin from Aloe vera (Aloe barbadensis Miller) shows specific characteristics, such as a natural low esterification degree and a high content of galacturonic acid [2]. This work aims at extracting pectin from Aloe vera and at evaluating its potentiality for the preparation of matrices to be used in regenerative medicine.

Materials and Methods: *Aloe vera leaves*. Fresh leaves of Aloe vera, kindly provided by Ambreck Pharmacy (Milan, Italy), were deprived of the green rind. The clear pulp was washed in distilled water to drain off the anthraquinone-rich sap. Fresh samples of the inner gel were stained with ruthenium red to selectively detect pectin distribution in the cell wall: briefly, pulp samples were immersed in a 2% (w/v) ruthenium red solution (30', RT), washed in distilled water and observed under a stereomicroscope (Nikon SMZ1000). *Aloe vera pectin*. Pectin was extracted from the inner gel of Aloe vera leaves by the following procedure: *a)* Separation of alchohol insoluble residues (AIRs) from the pulp to obtain the cell wall material; *b)* AIRs treatment for pectin extraction from the other cell wall material;*c)* Purification and isolation of the final product. The experimental parameters were varied to optimize the pectin extraction (e.g. yield). The extracted pectin was characterized by IR spectroscopy (Nicolet 6700) and the molecular weight estimated by viscosimetry. A spectrofotometric method was purposely developed to determine the esterification degree. *Aloe vera pectin gels*. The extracted pectin was washed with 70% (v/v) ethanol, dried at RT, dissolved in cool water (2% w/v) and added to 1,6% (w/v) $CaCl_2$ to produce gels. All steps were performed in a laminar flow box. Swelling and stability of the gels were evaluated in PBS at 37 °C. *In vitro* cell interaction was evaluated with osteosarcoma cell line SAOS-2 and the cell viability was evaluated by MTT test at 24h.

Results: Ruthenium red staining highlighted the distribution of pectin in the cell wall of the pulp (Fig. 1).

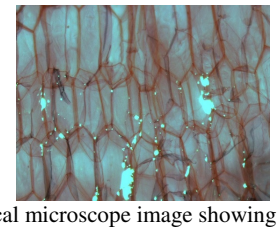

Figure 1. Optical microscope image showing the red staining of the pectin in the Aloe pulp cell wall.

Best conditions for pectin extraction were found in using 70% (v/v) ethanol and microwave pretreatment of the pulp for AIRs separation, treating AIRs with 50 mM sodium citrate aqueous solution (70°C, 2h), dialyzing the extract against distilled water and precipitating the product with isopropanol. By using these conditions, the final yield was increased from 4,2% to 29,11%. IR spectra of Aloe pectin (Fig. 2) showed homogalacturonan characteristic peaks in the 1200-1000 cm^{-1} region [3].

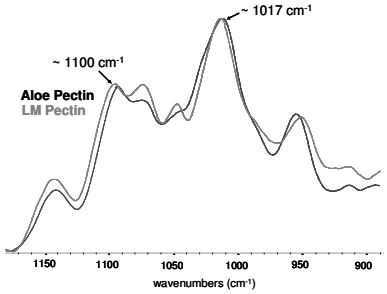

Figure 2. IR spectra of Aloe pectin and commercial low methoxy (LM) pectin (control).

The molecular weight of the extracted pectin was estimated ~ 120 kDa and the esterification degree value ~ 3%. The pectin gels increased 30 times their initial mass after 1h of incubation in PBS and the percentage of weight loss was about 15% after 7 days of incubation. The *in vitro* tests (Fig.3) showed that cell viability on Aloe pectin gels was comparable to the viability on tissue culture polystyrene (TCPS) and clearly improved with respect to the gels produced with LM commercial pectin

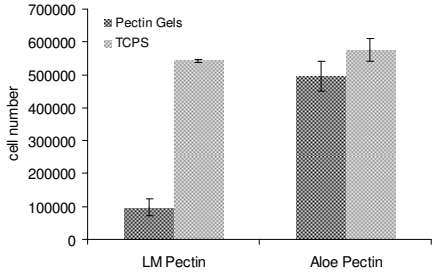

Figure 3. Cell viability on Aloe pectin and LM pectin gels. Data are compared with tissue culture polystyrene (TCPS).

Conclusions: In this work the extraction procedure of pectin from Aloe vera was optimized in terms of yield and physico-chemical properties of the final product. The obtained gels showed stability in PBS at 37 °C up to 7 days. Furthermore, preliminary *in vitro* tests demonstrated a much higher cytocompatibility of Aloe pectin compared to commercial LM pectin.

References:

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