Wound healing processes using *Punica granatum* Linn extracts incorporated in collagen based films

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Statement of Purpose: Collagen matrix is known to provide physical support for cellular proliferation. This characteristic qualifies it as an excellent material for wound healing. However, wound healing is a complex multifactorial process and the inflammation constitutes a part of the acute response of these events. The incorporation of bioactive substances in collagen matrix has been studied showing excellent results. *Punica granatum* Linn, a natural product tannin-rich, has been shown to possess several biological properties including antioxidant (free radical scavenging activity), antitumor, anti-inflammatory and antimicrobial. These activities make it a potential material for aid in wound healing process. We propose to incorporate this extract in the collagen films to ensure the release of active substances. The aim of this work was the development, characterization of collagen based films loaded with *Punica granatum* Linn extracts and the evaluation of wound healing processes using these films.

Methods: *Punica granatum* aqueous extract (PGE) was obtained by dynamics maceration. The characterization of PGE was carried out using HPLC-MS. It was determined the antioxidant potential by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Collagen (1%) membranes were produced with and without addition of PGE by casting process. The film characterization was performed by analysis of the mechanical properties, swelling degree, colorimetric assays, water vapor permeability, thermal analysis, infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The evaluation of the healing potential of membranes was performed on Swiss rats (n = 60,). Surgical wounds (8.0 mm diameter) were performed in 3 subgroups (n = 20): CTR (no treatment), COL (collagen dressing), and PGE (collagen dressing containing PGE). At 3, 7, 14 and 21 days of experiment, we calculated the wound retraction index (WRI). Histological sections were obtained and stained with hematoxylin-eosin and Sirius red. For statistical evaluation we used ANOVA post hoc Tukey and "T" test, considering significant $p < 0.05$.

Results: IC_{50} was 16.8±4.9 $\mu g.mL^{-1}$. The major compounds identified by HPLC-MS were punicalagin A and B, galic acid and elagic acid. The incorporation of PGE did not change in mechanical properties ($p>0.05$) compared to collagen films. We can observe an increase in PGE film thickness of 83.4% compared to COL films ($p<0.05$), confirmed by SEM. The water vapor transmission rate of PGE films was lower than COL films ($p<0.05$). PGE films presented higher swelling index in pH 7.2 than COL films ($p<0.05$). Changes in color were detected in L*, a*, b*, showing a yellowish tendency.

Table 1. Film characterization

Parameter	COL	PGE
Young modulus (MPa)	633.3±128.5	639.3±82.0
Elongation (%)	7.1±2.5	8.7±2.4
Maximal tension (MPa)	43.3±9.1	54.6±7.3
Permeability ($g.mm/d.m^2.KPa$)	329.1±28.5	12.1±1.3
Swelling index (%)	359.1±18.3	441.1±23.2

Concerning the wound healing assays, we can observe that PGE group presented retraction index higher than CTR and COL groups at days 3 ($p<0.01$) and 7 ($p<0.001$). At day 14, PGL group showed a higher retraction index compared to CTR group ($p<0.001$), however the results were similar to COL group ($p>0.05$). In histological sections, we can observe that the development and earlier stages maturation of granulation reaction in PGE group and an acute inflammatory response in CTR and COL in the initial phases (3 and 7 days). At day 14 and 21, PGE showed better pattern of tissue organization when compared to CTR and COL groups. Furthermore, PGE also exhibits the earlier formation of type III collagen net at day 3 and the faster substitution of collagen type III for type I at day 7. At day 14, we observed that the better architectural disposition of collagen fiber in PGE compared to other groups, however at day 21 PGE and COL presented similar morphology.

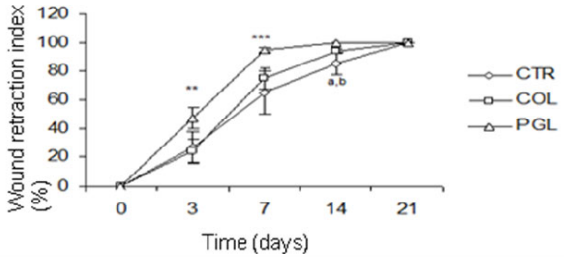

Figure 1. Wound retraction index.

Conclusions: The incorporation of aqueous PG extracts in collagen based film improves the wound healing process. The films also showed suitable physicochemical characteristics to wound healing application.

References:

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