Development of modified collagen based films containing red propolis extracts to wound healing application

Juliana C. Cardoso[1,2], Isana C. L. Souza[1], Marismar F. Nascimento[1], Luiz P. Costa[1,2], Ana L. S. Cristino[1], Ricardo L. C. Albuquerque Júnior[1,2].

[1]Universidade Tiradentes, Aracaju, Sergipe, Brazil

[2]Instituto de Tecnologia e Pesquisa, Aracaju, Sergipe, Brazil.

Statement of Purpose: The use of collagen based membranes as wound dressing has been demonstrated an important strategy to improvement the wound healing. However, collagen molecule is quickly degraded and crosslink reactions are needed to control the degradation rate and improve mechanical properties. The result of reaction of collagen with a reducing sugar are browning chromophores, fluorophores and protein cross-links. These glycated proteins promote an increase of material stiffness and resistance to enzymatic degradation. This approach can be used to improve the biomechanical properties of polymer. In order to improve the wound healing process, we have demonstrated that natural products used as anti-inflammatory/antioxidants agents showed important results. In this work, we used the red propolis, a new variety of Brazilian propolis , which one is flavonoid-rich. It is known for potential health benefit and is reported to possess valuable biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The goal of this work was the modification of collagen by Maillard reaction and characterization of film obtained from this molecule. We studied also these films containing red propolis extract (RPE) using in vivo model for wound healing assay.

Methods: The modification reaction was performed using collagen and maltodextrin (pH 7.2/25ºC). The hydroalcoholic extract of red propolis (RPE) was obtained by maceration. Collagen- (C) and modified collagen-based films (MC) were obtained by casting process. Films containing RPE were also performed using both polymers (CP and MCP). The films were characterized by mechanical analysis, water vapor permeability, swelling index, and colorimetric analysis. The wound healing potential of these films were assessed using in vivo model (n=100). Surgical wounds (8.0 mm diameter) were performed in 5 subgroups (n = 20): CTR (no treatment), C (collagen films), CP (collagen films with RPE), MC (modified collagen films) and MCP (modified collagen films with RPE). The results of wound retraction index and histological features of inflammatory reaction (hematoxylin/eosin) and remodeling process (Sirius red) were recorded at 3,7,14 and 21 days. The statistical analysis was performed by ANOVA post hoc Tukey test, considering significance $p < 0.05$.

Results: The mechanical properties results showed that the elongation (E) of MC films decreased when compared to C films. MC also presented lower water vapor permeability (WVP) and swelling index (SI) than C films. The chemical crosslinking decreased the possibilities the interactions water-polymer and changed these parameters. The color of the MC films was changed, demonstrating that the Maillard reaction occurred. The literature named this reaction as non enzymatic browning reaction. The incorporation of RPE in the formulations increased E of CP films when compared to C films. However MCP presented similar E to MC films. The same behavior was observed to WVP results, where CP showed lower WVP than C, but no significant difference was found between MC and MCP. The incorporation of RPE increased the swelling index for MCP compared to MC.

Table 1. Film characterization

Analysis	C	CP	MC	MCP
E (%)	$3.7\pm1.5^{a,c}$	6.4 ± 2.8^{a}	2.1 ± 1.5^{b}	$2.0\pm1.0^{b,c}$
T (MPa)	$47\pm17^{a,b}$	76 ± 26^{a}	37 ± 17^{b}	18 ± 6^{c}
YM (GPa)	1.3 ± 0.2^{a}	1.2 ± 0.3^{a}	2.0 ± 0.7^{a}	1.0 ± 0.4^{a}
WVP (g.mm/d. m^2.KPa)	$8.8\pm0,2^{a}$	6.2 ± 0.2^{b}	7.3 ± 0.1^{c}	7.1 ± 0.1^{c}
SI (%)	313 ± 23^{a}	345 ± 19^{a}	126 ± 6^{b}	258 ± 35^{c}

E (elongation); T (tension); YM (Young modulus); WVP (water vapor permeability); SI (swelling index). Different letter in the same line represents values statistically different ($p<0.05$).

In biological assays, the results showed that at day 14, the wound retraction index (WRI) presented significant difference between CMP ($p<0.05$) compared to others groups, showing an improvement in wound healing process. It was observed that in CP and CMP the formation of a complete thin epithelium. Amorphous aggregates indicating that the films keep present in the wound were observed in CM e CMP films at histological sections at day 7.

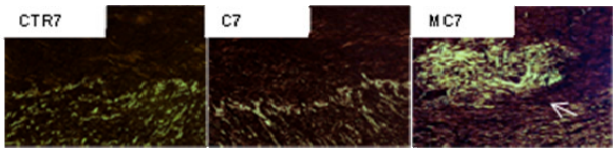

Figure 1. Histological section (Sirius red stained). Modified collagen film (white arrow in MC7) at day 7.

Conclusions: The results showed that the collagen modification changed the biodegradability of film, as well the physicochemical parameters. The incorporation of RPE in films promoted the improvement of wound healing process using *in vivo* model.

References:

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