Assessment of the clinical, cellular, and molecular effects of Royalactin-Royal Jelly on the healing of Wagner Grade II diabetic foot ulcers: A phase II, double-blind, randomized controlled trial.

Valoración de los efectos clínicos, celulares y moleculares de la Royalactina-Jalea Real en la cicatrización de úlceras de Pie diabético Wagner II. Ensayo clínico controlado doble ciego fase II**.**

Completed September 2025

Abstract

Objective: To assess the clinical, cellular, and molecular effects of Royalactin on the healing of Wagner Grade II diabetic foot ulcers compared to a placebo.

Materials and Methods: A total of 20 observations were derived from dividing 10 wounds in diabetic patients recruited from a chronic wound and diabetic foot management center at a hospital in Northeastern Mexico for this double-blind randomized controlled trial. Each wound was divided, treated with standard techniques three times per week, and randomized to receive either placebo or Royalactin gel on each side for 4 weeks. At baseline and study completion, wound area (cm^2) was measured, and biopsies were taken, stained, and labeled to classify epithelial characteristics, inflammatory response, fibroblast presence, angiogenesis, collagen content, and the expression and intensity of VEGF and EGFR.

Results: In the clinical evaluation, both groups showed a statistically significant reduction in wound area; however, this reduction was greater in the Royalactin group (from 6.3 to 3.4 cm^2, $p = 0.003$) compared to the control group (from 7.8 to 3.9 cm^2, $p = 0.036$). Regarding cellular activity, no statistical significance was reached, but the Royalactin group showed an increase in cases with marked infiltration in the granulation tissue (10% to 50%), an increase in fibroblasts with moderate distribution (20% to 60%), an increase in cases with moderate angiogenesis (30–40%), and an increase in collagen content. At the molecular level, both groups showed an increase in VEGF expression, with a higher proportion in the Royalactin group (18% increase: from 32% to 50%) than in the control group (4.5% increase: from 39% to 43.5%), approaching statistical significance ($p = 0.072$), with an increase in expression

intensity from mild to moderate (10% to 30%). No EGFR expression was detected in either group at baseline or after 4 weeks.

Conclusions: A significant reduction in average wound area was observed, accompanied by favorable modifications in key histological parameters and a trend toward increased VEGF expression, particularly in the Royalactin-treated group. EGFR expression was not detected (0 in all evaluated cases), suggesting that this pathway was not activated under the study conditions.

Introduction.

The prevalence of diabetic foot ulcers (DFU) ranges from 6% to 15% and accounts for nearly 20% of hospital admissions among diabetic patients, who face a 15-fold higher risk of amputation compared to non-diabetics (1,2). Risk factors for DFU include male gender, disease duration exceeding 10 years, high BMI, and comorbidities such as neuropathy, chronic arterial occlusive disease, and infection. Other factors include elevated glycated hemoglobin levels (3-6), foot deformities and instability (7,8), high plantar pressures, and inappropriate foot self-care habits (9-11). Management is aimed at achieving ulcer closure as quickly as possible, based on four principles: optimizing perfusion, adequate debridement, infection control, and balanced pressure redistribution on the affected foot. To these ends, various treatments are used, including the application of topical agents and dressings made of diverse materials; honey with Royal Jelly (RJ) is one of the oldest among them.

The re-epithelializing effects of RJ have been reported, such as the stimulation of fibroblast migration to the injury site through a dose-dependent mechanism that accelerates the healing process (12), as well as the stimulation of keratinocyte production and migration (13). Among the components of RJ, **Royalactin** stands out as the most significant peptide (14,15). This protein activates the signaling pathway for the expression of the **Epidermal Growth Factor Receptor (EGFR)** in bee larvae, leading to epigenetic changes and the development of larger body size compared to worker bees (approximately double), accelerated growth, and life extension (up to three years compared to an average of three weeks in worker bees) (16).

Additionally, a study demonstrated that this molecule also extends lifespan in the nematode *Caenorhabditis elegans* through the expression of epidermal growth factor and its receptor;

another described property is antimicrobial action (17). The activation of **EGFR** is one of the most critical mechanisms in healing processes and is a key point in cellular signaling to induce the migration and division of fibroblasts, keratinocytes, and endothelial cells in wounds. Furthermore, it activates epithelial-mesenchymal transition processes at the wound periphery, participates in cellular homeostasis, and stimulates angiogenesis. The synergistic activity of these effects induces accelerated granulation, re-epithelialization, wound contraction, and dermal maturation, achieving a healing rate three times faster than conventional methods, especially in diabetic foot ulcers. Controlled clinical trials have demonstrated that its application in wounds promotes the speed of healing (18,19).

Objective

The objective of this study is to evaluate the clinical, cellular, and molecular effects of Royalactin as an adjunct to standard treatment, compared to standard treatment alone, in the management of **Wagner Grade II** diabetic foot ulcers by assessing wound dimensions, cellular behavior, and growth factor expression.

Study Design

This is a randomized, double-blind, prospective, experimental, longitudinal, and comparative controlled clinical trial.

Materials and methods

Ethical Considerations The study was conducted in compliance with Good Clinical Practice (GCP) guidelines. All procedures adhered to the ethical standards of the Research and Bioethics Committee of the XXXXXXXXXX. the Declaration of Helsinki, and the General Health Law. Protocols were strictly followed, data were handled with rigorous confidentiality, and informed consent was obtained from all participants.

Inclusion Criteria Adult patients with Diabetes Mellitus undergoing treatment for foot and ankle wounds were included. Eligibility criteria required wounds larger than 4 cm^2, with more than 80% granulation tissue, involving skin, fat, or ligaments without bone involvement, and non-infected (**Wagner Grade II**) (20). Additional requirements included a duration of more than 2 months, an ankle-brachial index (ABI) greater than 0.5, the signing of the informed consent form, and the ability to attend dressing changes three times per week for 4 weeks.

Experimental Agents and Randomization The FEMSA Biotechnology Center laboratory produced 20 tubes (50 ml) containing gold nanoparticles and emulsifiers. Ten tubes were formulated with Royalactin at 0.2 g/L, while the other 10 served as the control without the peptide. The laboratory randomized, numbered, and paired the tubes, providing a list for paired application.

Treatment Protocol Standardized dressing changes were performed three times per week for 4 weeks at the Chronic Wound, Diabetic Foot, and Hyperbaric Medicine Center of XXXXX. Each wound was first irrigated with sterile water and dried with sterile gauze. The wound was then divided (or the division was remarked) using black ink on the skin, and a 0.5 cm strip of Hypafix (BSN medical GmbH, ESSITY) was applied. A circular label was placed on the perilesional skin to identify the tube number (bottom), patient ID (top-right), and dressing change number (top-left).

Standard photographs were taken (Camera+ App, iPhone 15 Pro Max). The area was gently debrided three times with sterile gauze moistened with 0.12% Chlorhexidine (Clorhexi-Clean®, PISA). Ketanserin 2% gel (Sufrexal®, Janssen) was applied to both sides, followed by the application of the corresponding gel (Royalactin or placebo) to each designated wound section. Finally, a primary dressing of sodium carboxymethylcellulose hydrofiber and regenerated cellulose fiber (Aquacell extra®, Convatec) was applied, protected by a secondary adhesive border gauze dressing (Border gauze®, Medline).

Data Collection and Histological Analysis During the first and last dressing changes (sessions 1 and 13), photographs were taken to measure the area of each side in cm^2 (IMITO measure App, iPhone 15 Pro Max). Additionally, 3 mm punch biopsies of the granulation tissue were obtained from the distal edge of each side.

Biopsy samples were sectioned, labeled, and stained using Hematoxylin and Eosin (H&E), Masson's Trichrome, and immunohistochemistry (IHC) for the expression of:

- **CD31** (Roche)
- **Vascular Endothelial Growth Factor (VEGF)** (Biocare)
- **Epidermal Growth Factor Receptor (EGFR)** (Cell Marque) (Figure 1 A-F).

Statistical Analysis and Evaluation Results from sessions 1 and 13 were evaluated. Wound size was recorded in cm^2. Epithelialization, polymorphonuclear neutrophils (PMN), fibroblasts, angiogenesis, and collagen were scored according to a semi-quantitative histological scale for wound assessment (Table 1) (21). The percentage of tissue expressing VEGF and EGFR, as well as the intensity of expression (scored 0 to 3+), were reported separately (Figure 1 G-I). Data were

compared at baseline and at four weeks, both as a total cohort and by comparing the Control vs. Royalactin groups; individual variables were also analyzed.

Results

A total of 20 clinical and histological observations were included (2 per wound), corresponding to Wagner Grade II DFUs treated over a 4-week follow-up period. The study population consisted of 3 women and 7 men, with a mean age of 55.1 years, a mean duration of Diabetes Mellitus of 15.5 years, 3 patients with arterial hypertension, and 4 with mild peripheral arterial disease. Wounds had a mean evolution time of 6.7 months and a mean length of 8.6 cm. At baseline, 95% of the wounds showed granulation tissue, all presented mild serosanguinous exudate (staining), and most reported low-intensity pain and localized edema. All wounds presented erythema (half < 2 cm and the other half > 2 cm).

All 20 observations were compared between baseline parameters and those obtained at the end of treatment (Table 2). A significant reduction in the mean wound area was observed, decreasing from 7.0 cm^2 (SD: 6.2) to 3.7 cm^2 (SD: 3.9) ($p = .034$). Regarding epithelialization, the lowest value predominated; thickness of cut edges started at 16 cases (80%) and decreased to 13 (70%), cell migration ≥50% remained unchanged (5%), while excision union increased from 5% to 10% and keratinization from 10% to 15% ($p = .865$). The PMNL analysis showed a trend toward increased evaluation scores after 4 weeks ($p = .524$), with a decrease in PMNL cases within the dermis/granulation tissue (D/GT) from 45% to 30%. Conversely, moderate infiltrate increased from 25% to 40%, and marked infiltrate in the same area rose from 10% to 35%, consistent with an inflammatory peak associated with the active repair phase. Fibroblast presence showed qualitative improvement, with an increase in moderate infiltrate cases in granulation tissue (GT) from 25% to 45%, with no marked infiltrate observed in any phase ($p = .520$). Angiogenesis evolved favorably: cases without vascular evidence disappeared (10% to 0%), moderate angiogenesis in GT increased (20% to 30%), and marked angiogenesis appeared in 5% of cases ($p = .549$). Regarding collagen synthesis, a statistically significant transition toward increased

synthesis was observed (p = .008), moving from absence (15% to 0%) and minimal collagen (45% to 40%) toward mild (40% to 25%) and moderate (0% to 35%) presence in GT by the end of treatment. At the molecular level, an increase in VEGF expression was identified, rising from 35.5% (SD: 20.8) to 46.75% (SD: 18.22) (p = .344). Furthermore, VEGF 1+ expression intensity increased from 50% to 65%, while 2+ expression remained constant (35%), and extreme values (0 and 3+) disappeared, indicating a more uniform response ($p = .396$). In contrast, no EGFR expression was detected (0 in all evaluated cases), suggesting this pathway was not activated under study conditions.

In the initial assessment of the 10 lesions selected as controls and the 10 selected for Royalactin application, results showed that clinical, histological, and molecular characteristics were generally comparable, with no statistically significant differences in most variables, indicating adequate randomization (Table 3). Mean wound area was slightly larger (7.8 cm^2, SD: 7.1) in Without Royalactin lesions compared to those selected for Royalactin (6.3 cm^2, SD: 5.4), with no statistically significant difference (p = .684). Regarding epithelialization, thickness in cut edges predominated in both groups (70% vs. 90%), being higher in the Royalactin group, which also showed an exclusive presence of cell migration ≥50%. Keratinization and excision union were observed in the Without Royalactin sections (p = .334). Regarding the PMN inflammatory response, a higher proportion of mild infiltrate in D/GT was seen in Royalactin sections (70% vs. 20%), while control sections presented a higher proportion of moderate infiltrate in GT (40% vs. 10%) (p = .125). Fibroblast distribution was nearly identical, predominantly mild or moderate in GT (p = .848). Angiogenesis was mainly mild in GT, with higher values in the Royalactin group (80% vs. 50%), while moderate angiogenesis was more frequent in the control (30% vs. 10%) (p = .628). For collagen, sections with minimal and mild presence in GT predominated (70% and 20% in control vs. 30% and 50% in Royalactin); both showed similar absence values (p = .484).

Molecularly, VEGF expression was similar (Mean: 39% SD: 24 vs. 32% SD: 16, p = .470), with a slight predominance (7%) in control sections. However, expression intensity showed significant differences (p = .014); 1+ intensity was observed in 80% of Royalactin sections versus 20% in the control group, while 2+ expression was more frequent in the latter (60%). No cases of maximum expression (3+) were identified. No EGFR expression was detected at baseline.

After four weeks of treatment, clinical, histological, and molecular effects were compared between groups (Table 4). Mean wound area was slightly smaller with Royalactin (3.4 cm^2, SD: 4.2) versus control (3.9 cm^2, SD: 3.8) (p = .677). Histologically, the initial biopsy site (GT at the wound edge) had epithelialized in all observations by week 4; thus, end-of-study biopsies were taken from the new wound edge. For epithelialization, more cases of thickness in cut edges were seen in the Royalactin group (80% vs. 50%), with the latter showing a single case of cell migration ≥50% and another with keratinization. Keratinization and excision union were slightly more frequent (20%) in control sections (p = .471). In the PMNL infiltrate assessment, both groups showed similar patterns, predominantly mild, moderate, and marked in D/GT ($ = .727), with one case of absent infiltrate in the Royalactin group. Fibroblasts were similarly distributed (mild and moderate in GT, p = .727), with the highest moderate distribution seen in Royalactin (50%). Angiogenesis was very similar (p = .999), predominantly mild in GT (50% vs. 60%); marked angiogenesis was only observed in one control section. Higher collagen percentage was found in the minimal assessment of control sections (50%) compared to Royalactin (30%); however, Royalactin showed higher mild (30% vs. 20%) and moderate (40% vs. 30%) ratings. Molecularly, VEGF expression was higher in Royalactin (Mean: 50%, SD: 17) versus control (Mean: 43.5%, SD: 19.7) (p = .440). VEGF intensity was similar in all sections, predominantly 1+ (60% vs. 70%) and 2+ (40% vs. 30%) (p > .999). No EGFR expression was detected.

When comparing baseline versus 4-week effects separately for both groups (Table 5), it was observed that both groups showed a decrease in wound area, which was more pronounced in the Royalactin group (from 6.3 SD: 5.4 to 3.4 cm^2 SD: 4.2, $p = .089$) compared to the control group (from 7.8 SD: 7.1 to 3.9 cm^2 SD: 3.8, $p = .185$) (Graph 1). Histological results followed a similar comparison pattern (Graph 2). In epithelialization, the lowest rating predominated and decreased by week 4 in both control (70% to 60%) and Royalactin (90% to 80%) groups ($p = .820$ and $p = .999$ respectively). PMN distribution in control sections showed an increase in mild infiltrate in D/GT (20% to 40%, $p = .836$). In Royalactin sections, mild D/GT infiltrate decreased (70% to 20%), while moderate (10% to 40%) and marked (10% to 30%) infiltrate increased, showing a trend ($p = .168$). Fibroblasts showed a discrete progression in controls ($p = .999$), while Royalactin sections highlighted a trend ($p = .187$) toward moderate GT distribution (20% to 50%). Angiogenesis was very similar in controls ($p = .999$), whereas Royalactin sections showed a progression from mild GT (80% to 60%) to moderate (10% to 30%) ($p = .714$). Collagen increased in both groups, reaching 0% absence at week 4 and progressing to moderate presence in controls (0% to 30%, $p = .276$) and further in Royalactin (0% to 40%, $p = .071$). Molecular assessment showed a significant increase ($p = .089$) in VEGF expression percentage in Royalactin sections (32% SD: 16 to 50% SD: 17), higher than in controls (39% SD: 24 to 43.5 SD: 19.7, $p = .795$) (Graph 3). VEGF 1+ intensity increased in controls and decreased in Royalactin, while 2+ increased in Royalactin ($p = .170$ for controls vs. $p = .582$ for Royalactin). No EGFR expression was detected.

Finally, a linear regression between wound area (cm^2) and VEGF expression (%) at baseline found a negative correlation in both control ($R^2 = 0.38$, $p = .057$) and Royalactin ($R^2 = 0.16$, $p = .253$) groups. This suggests that higher VEGF levels may be associated with smaller lesions and a more active tissue environment. At 4 weeks, the correlation remained negative in the control

group ($R^2 = 0.24$, $p = .153$), but was completely lost in the Royalactin group ($R^2 = 0.04$, $p = .558$) (Graph 4).

Discussion

The baseline evaluation of both control and Royalactin-treated wound areas demonstrated that, overall, the clinical, histological, and molecular characteristics were comparable, with no statistically significant differences across most variables. This indicates successful randomization, with the sole exception of a higher baseline intensity of VEGF expression in the control sections.

Our standard wound care management effectively promoted healing, as evidenced by a statistically significant reduction in all wound areas ($p = .034$). This reduction was more pronounced in the Royalactin group ($p = .089$), a finding that aligns with previous studies describing the re-epithelializing and pro-angiogenic properties of Royal Jelly (12–14). This result is clinically significant, as an increased rate of wound closure is associated with lower rates of infectious complications and a reduced risk of amputation in patients with diabetic foot ulcers.

In our study, the wound edges where initial biopsies were taken achieved complete re-epithelialization in all cases. These changes may reflect a more favorable tissue environment for keratinocyte migration, consistent with the findings of Lin et al., who documented the mitogenic effect of royal jelly-derived proteins in human skin models (13).

In the Royalactin-treated sections, unlike the control group, a trend toward an increase in moderate infiltrate within the granulation tissue was observed ($p = .168$ vs. $p = .836$). This can be interpreted as an intensification of the local inflammatory response a migratory effect previously reported (14) and associated with an active regeneration phase in the absence of clinical infection. Additionally, an increase in fibroblast presence to moderate levels in the granulation tissue was observed ($p = .187$ vs. $p = .999$). Linked to a significant increase in collagen ($p = .071$ vs. $p = .276$), this likely suggests the stimulation of cellular migration. Angiogenesis also exhibited a discreetly favorable pattern ($p = .714$ vs. $p = .999$), with an increase in cases showing moderate angiogenesis.

At the molecular level, Royalactin-treated sections showed a statistically significant higher percentage of VEGF expression ($p = .0379$ vs. $p = .759$). While the intensity of VEGF expression decreased in the control group, it increased in the Royalactin-treated sections ($p = .582$ vs. $p = .170$).

This finding is coherent with literature linking Royalactin to enhanced cell survival and growth in *Caenorhabditis elegans* and tissues exposed to hypoxia via the modulation of EGFR and VEGF (17,18). In our study, immunohistochemical activation of EGFR was not detected in human tissue, possibly due to differences in methodological sensitivity or distinct transcriptional activation requirements in humans.

Conclusiones

The present study explored the clinical, histological, and molecular effects of Royalactin, a bioactive protein derived from royal jelly, on the healing process of Wagner Grade II diabetic foot ulcers. Our findings demonstrate that, after four weeks of treatment, a reduction in the mean wound area was achieved, accompanied by favorable modifications in key histological parameters and an increase in VEGF expression, particularly in the Royalactin-treated group. These results justify the development of Phase III clinical trials with greater statistical power, as well as complementary studies to explore additional molecular pathways.

EGFR expresión was not detected (0 in all evaluated cases), suggesting that this pathway was not activated under the study conditions.

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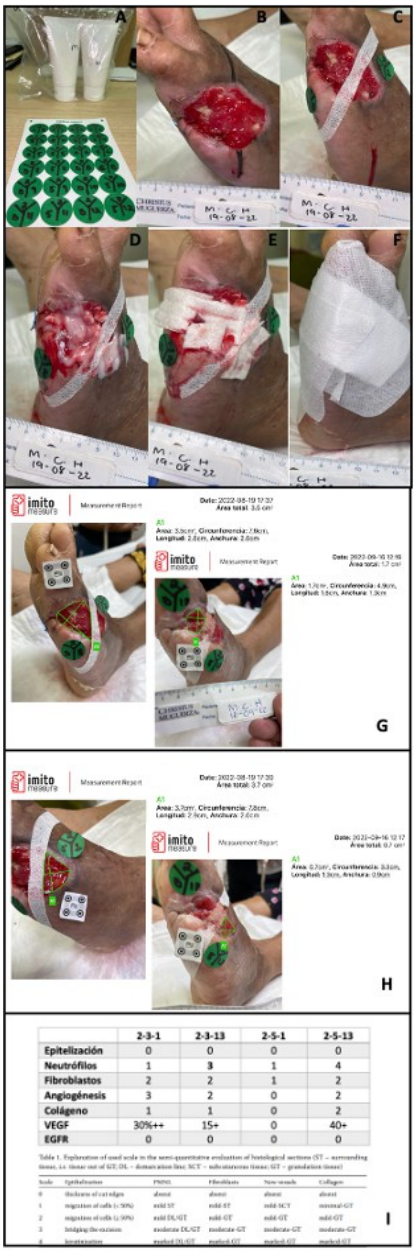

FIGURE 1. **A** Jars and labels; **B** Wound division; **C** Marking and biopsies; **D** Applied gel; **E** Primary hydrofiber dressing; **F** Secondary absorbent dressing; **G** Patient 2, jar 3, treatments 1 and 13; **H** Patient 2, jar 5, treatments 1 and 13; **I** Semiquantitative table of cellular and molecular findings.

Table 1. Explanation of used scale in the semi-quantitative evaluation of histological sections (ST – surrounding tissue, i.e. tissue out of GT; DL – demarcation line; SCT – subcutaneous tissue; GT – granulation tissue)

Scale	Epithelization	PMNL	Fibroblasts	New vessels	Collagen
0	thickness of cut edges	absent	absent	absent	absent
1	migration of cells (< 50%)	mild ST	mild-ST	mild-SCT	minimal-GT
2	migration of cells (≥ 50%)	mild DL/GT	mild-GT	mild-GT	mild-GT
3	bridging the excision	moderate DL/GT	moderate-GT	moderate-GT	moderate-GT
4	keratinization	marked DL/GT	marked-GT	marked-GT	marked-GT

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Table 2. Comparison of general variables **N = 20[1]**

Variables	**Base line**	**4 weeks**	**p**
WOUND AREA (cm²)	7.0 (6.2)	3.7 (3.9)	0.034
Epithelization			0.865
Thickness of cut edges	16 (80%)	14 (%)	
Cell migration (≈ 50%)	0 (0%)	0 (0%)	
Cell migration (≥ 50%)	1 (5.0%)	1 (5.0%)	
Excision union	1 (5.0%)	2 (10%)	
keratinization	2 (10%)	3 (15%)	
PMNL			0.524
absent	1 (5.0%)	1 (5.0%)	
mild in ST	2 (10%)	0 (0%)	
Mild in DL/GT	9 (45%)	6 (30%)	
moderate in DL/GT	5 (25%)	8 (40%)	
Moderate in DL/GT	3 (15%)	5 (25%)	
Fibroblasts			0.520
absent	1 (5.0%)	0 (0%)	
mild in ST	6 (30%)	5 (25%)	
mild in GT	8 (40%)	6 (30%)	
Moderate in GT	5 (25%)	9 (45%)	
Significant in GT	0 (0%)	0 (0%)	
Angiogenesis			0.549
absent	2 (10%)	0 (0%)	
mild in SCT	1 (5.0%)	2 (10%)	
mild in GT	13 (65%)	11 (55%)	
moderate in GT	4 (20%)	6 (30%)	
significant in GT	0 (0%)	1 (5.0%)	
Collagen			0.008
Absent	3 (15%)	0 (0%)	
minimal in GT	9 (45%)	8 (40%)	
mild in GT	8 (40%)	5 (25%)	
moderate in GT	0 (0%)	7 (35%)	
significant in GT	0 (0%)	0 (0%)	
VEGF (%)	35.5 (20.8)	46.75 (18.22)	0.344
VEGF (+++)			0.396
0	2 (10%)	0 (0%)	
1	10 (50%)	13 (65%)	
2	7 (35%)	7 (35%)	
3	1 (5.0%)	0 (0%)	
EGFR			
0	20 (100%)	20 (100%)	
	[1] Mean (DE) o Frequency		

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Table 3 Comparation of Royalactin group at baseline measurment

Variables	Without Royalactin N = 10[1]	Royalactin Group N = 10[1]	P
WOUND AREA(cm²)	7.8 (7.1)	6.3 (5.4)	0.684
Epitheliazation			0.334
Thickness of cut edges	7 (70%)	9 (90%)	
Cell migration (≈ 50%)	0 (0%)	0 (0%)	
Cell migration (≥ 50%)	0 (0%)	1 (10%)	
Excision union	1 (10%)	0 (0%)	
keratinization	2 (20%)	0 (0%)	
PMNL			0.138
absent	1 (10%)	0 (0%)	
Mild in ST	1 (10%)	1 (10%)	
Mild in DL/GT	2 (20%)	7 (70%)	
Moderate in DL/GT	4 (40%)	1 (10%)	
significant in DL/GT	2 (20%)	1 (10%)	
Fibroblasts			0.848
absent	1 (10%)	0 (0%)	
Mild in ST	3 (30%)	3 (30%)	
Mild in GT	3 (30%)	5 (50%)	
moderate in GT	3 (30%)	2 (20%)	
Significant in GT	0 (0%)	0 (0%)	
Angiogenesis			0.628
absent	1 (10%)	1 (10%)	
Mild in SCT	1 (10%)	0 (0%)	
Mild in GT	5 (50%)	8 (80%)	
moderate in GT	3 (30%)	1 (10%)	
significant in GT	0 (0%)	0 (0%)	
Collagen			0.484
absent	1 (10%)	2 (20%)	
minimal in GT	6 (60%)	3 (30%)	
mild in GT	3 (30%)	5 (50%)	
moderate in GT	0 (0%)	0 (0%)	
significant in GT	0 (0%)	0 (0%)	
VEGF (%)	39 (24)	32 (16)	0.470
VEGF (+++)			0.014
	1 (10%)	1 (10%)	
+	2 (20%)	8 (80%)	
++	6 (60%)	1 (10%)	
+++	1 (10%)	0 (0%)	
++++	0 (0%)	0 (0%)	
EGFR			
0	10(100%)	10(100%)	

[1] Mean (IC) or Frecuency

[2] Fisher's exact test; Wilcoxon rank sum exact test; Wilcoxon rank sum test

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Table 4 Comparation of Royalactin group at 4 weeks

Variables	**Without Royalactin N = 10[1]**	**Royalactin N = 10[2]**	**P**
WOUND AREA (CM²)	3.9 (3.8)	3.4 (4.2)	0.677
Epithelialization			.471
Thickness of cut edges	6(60%)	8 (80%)	
Cell migration (≈ 50%)	0 (0%)	0 (0%)	
Cell migration(≥ 50%)	0 (0%)	1 (10%)	
Excision union	2(20%)	0 (0%)	
keratinization	2 (20%)	1 (10%)	
PMNL			0.727
absent	0 (0%)	1 (10%)	
Mild in ST	0 (0%)	0 (0%)	
mild in DL/GT	4 (40%)	2 (20%)	
moderate in DL/GT	4 (40%)	4 (40%)	
significant in DL/GT	2 (20%)	3 (30%)	
Fibroblasts			0.727
absent	0 (0%)	0 (0%)	
Mild in ST	2 (20%)	3 (30%)	
Mild in GT	4 (40%)	2 (20%)	
moderate in GT	4 (40%)	5 (50%)	
significant in GT	0 (0%)	0 (0%)	
Angiogenesis			0.999
absent	0 (0%)	0 (0%)	
Mild in SCT	1 (10%)	1 (10%)	
Mild in GT	5 (50%)	6 (60%)	
moderate in GT	3 (30%)	3 (30%)	
significant in GT	1 (10%)	0 (0%)	
Collagen			0.735
absent	0 (0%)	0 (0%)	
minimal in GT	5 (50%)	3 (30%)	
mild in GT	2 (20%)	3 (30%)	
moderate in GT	3 (30%)	4 (40%)	
significant in GT	0 (0%)	0 (0%)	
VEGF (%)	43.5 (19.7)	50 (17)	0.440
VEGF (+++)			0.999
	0 (0%)	0 (0%)	
+	6 (60%)	7 (70%)	
++	4 (40%)	3 (30%)	
+++	1 (10%)	0 (0%)	
++++	0 (0%)	0 (0%)	
EGFR			
0	10(100%)	10(100%)	

[1] Mean (IC) or Frecuency

[2] Fisher's exact test; Wilcoxon rank sum exact test; Wilcoxon rank sum test

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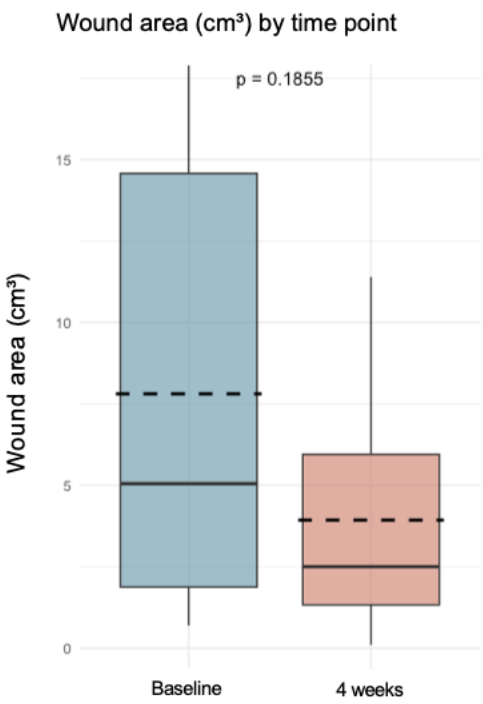
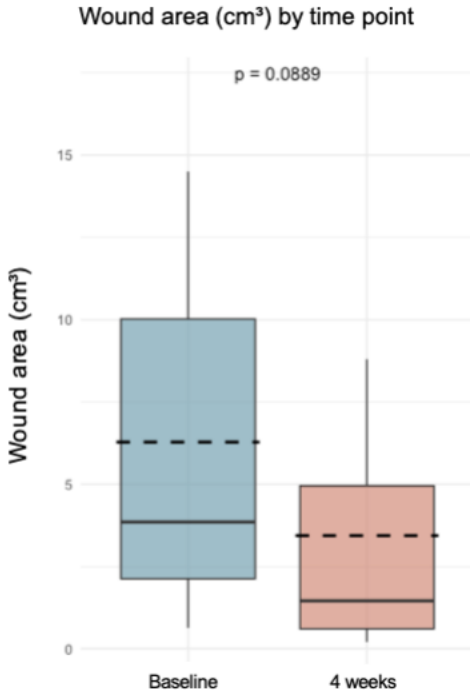
Without royalactin



With royalactin



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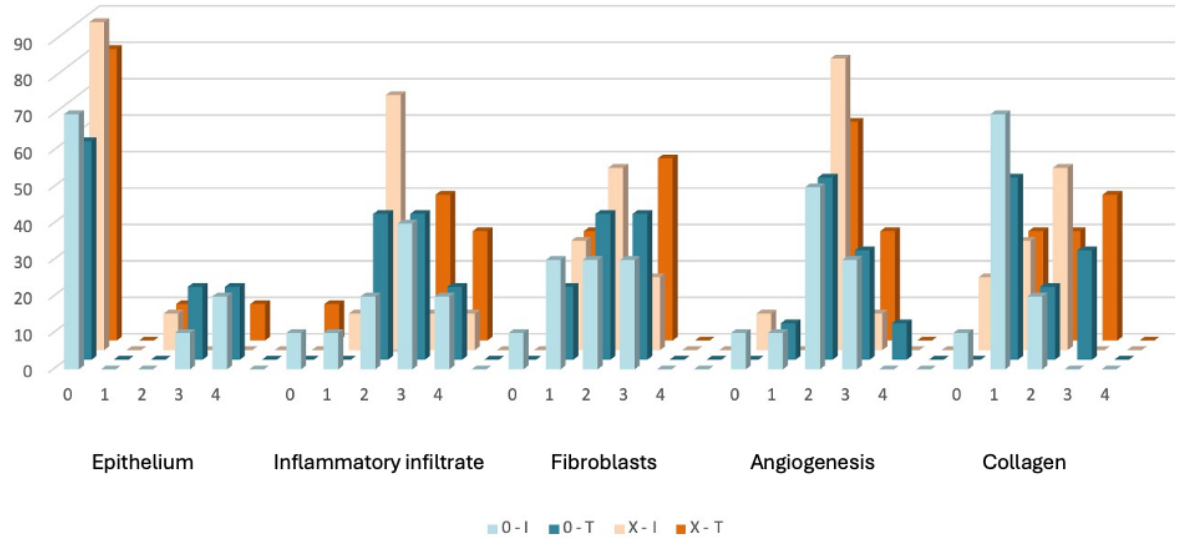
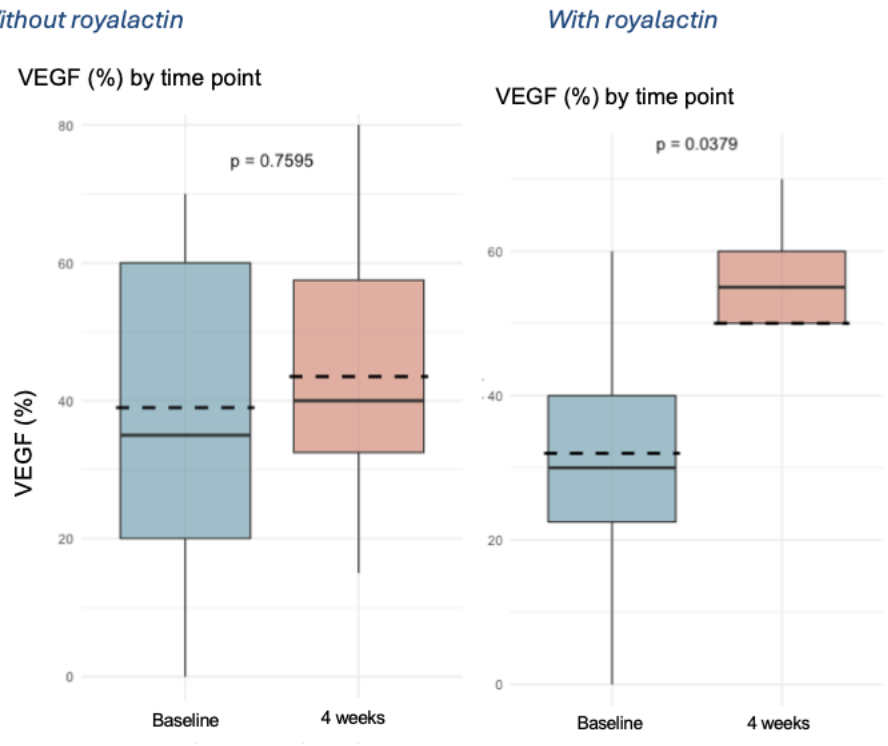
Graph 1

Wound area in the control group decreased from 7.8cm^2 (SD: 7.1) to 3.9cm2 (SD: 3.8) (p = 0.185), and in the Royalactin group from 6.3cm^2 (SD: 5.4) to 3.4cm^2 (SD: 4.2) (p = 0.089).



Graph 2 – Results of the semiquantitative histological wound assessment scale, comparing control groups at baseline (O-I) and study endpoint (O-T) versus Royalactin-treated groups at baseline (X-I) and study endpoint (X-T).



Graph 3: Percentage of vascular endothelial growth factor (VEGF) expression in control sections (39% SD: 24 to 43.5% SD: 19.7) ($p = 0.795$) and in Royalactin-treated sections (32% SD: 16 to 50% SD: 17) ($p = 0.088$).

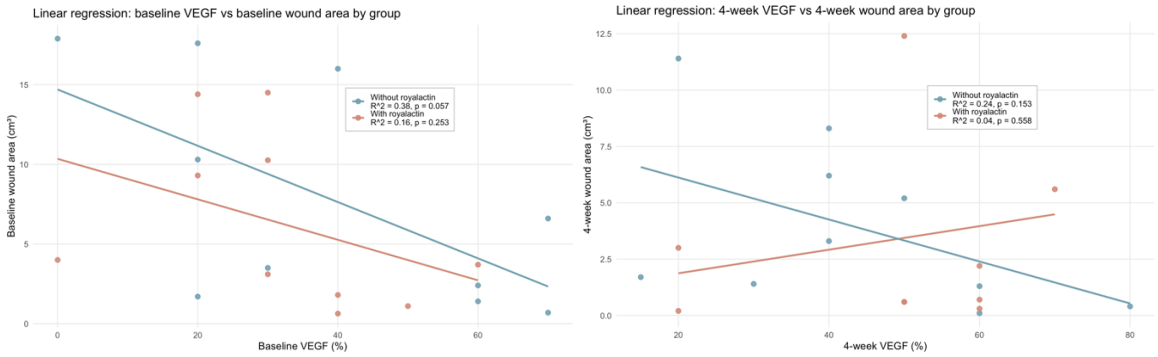


Figure 4 Correlation between wound area and VEGF expression. Linear regression analysis comparing wound area and VEGF percentage at baseline (left) and after 4 weeks of treatment (right) for both the control and Royalactin-treated groups.

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