

The Impact of Lactobacillus-Rich Probiotic Nutrition on the Pharmacological Efficacy of Clotrimazole Delivered via Mucoadhesive Thermosensitive Hydrogels in Human Subjects with Recurrent Candidiasis

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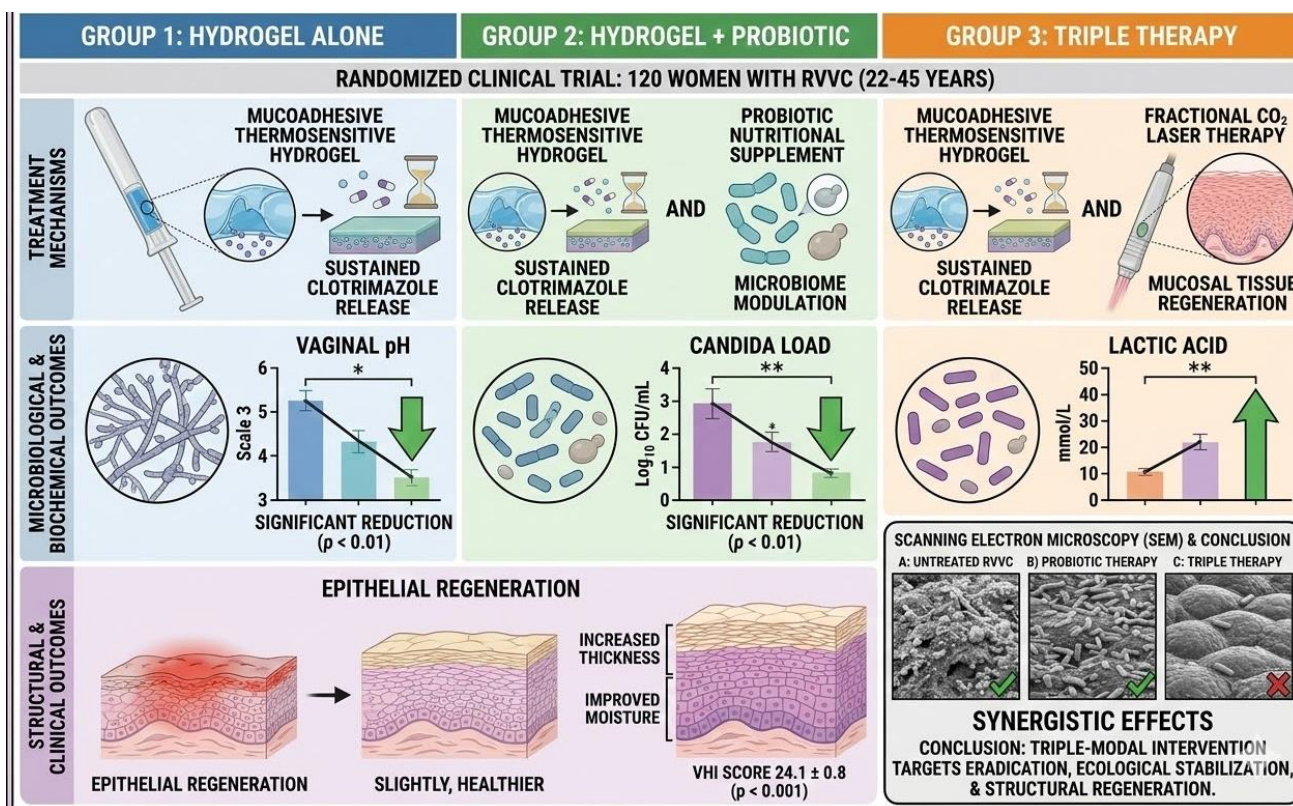
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Graphical Abstract



Abstract

Background: Recurrent vulvovaginal candidiasis (RVVC) is a persistent infectious and inflammatory condition associated with microbial dysbiosis, biofilm-mediated fungal resistance, and impaired mucosal structural integrity. Emerging therapeutic approaches emphasize multi-layered interventions integrating pharmacological delivery systems, microbiome modulation, and tissue regeneration strategies. **Objective:** This study evaluated the impact of Lactobacillus-rich probiotic nutrition on the pharmacological efficacy of clotrimazole delivered via mucoadhesive thermosensitive hydrogels, with adjunct fractional CO₂ laser therapy in human subjects with recurrent candidiasis. **Methods:** A randomized comparative clinical design was conducted involving 120 women aged 22–45 years diagnosed with RVVC. Participants were allocated into three groups receiving clotrimazole-loaded mucoadhesive

thermosensitive hydrogel therapy alone, hydrogel therapy combined with probiotic nutritional supplementation, or triple therapy including probiotic nutrition and fractional CO₂ laser treatment. Clinical, microbiological, biochemical, and structural outcomes were evaluated over 12 weeks of treatment followed by 6 months of recurrence monitoring. **Results:** The triple-therapy group demonstrated superior clinical and biochemical outcomes, including significant reduction in vaginal pH, decreased *Candida* load, and enhanced lactic acid concentration ($p < 0.01$). Structural assessment revealed marked improvement in vaginal epithelial thickness, moisture retention, and Vaginal Health Index scores ($p < 0.001$). Scanning electron microscopy confirmed restoration of epithelial surface architecture and minimal fungal biofilm presence in the triple-therapy group. **Conclusion:** Lactobacillus-rich probiotic nutrition significantly enhances the pharmacological effectiveness of clotrimazole delivered through mucoadhesive thermosensitive hydrogels. The integration of microbiome restoration and fractional CO₂ laser therapy produces synergistic therapeutic effects by targeting fungal eradication, microbial ecological stabilization, and mucosal structural regeneration. Triple-modal intervention represents a promising advanced therapeutic strategy for recurrent vulvovaginal candidiasis; however, large-scale multicenter randomized trials are required to validate long-term safety and clinical efficacy.

Keywords: Recurrent vulvovaginal candidiasis; Lactobacillus probiotics; Mucoadhesive thermosensitive hydrogel; Clotrimazole delivery; Fractional CO₂ laser therapy; Vaginal microbiome restoration; Biofilm inhibition; Mucosal regeneration; Triple-modal therapy; Functional probiotic nutrition.

Introduction

Recurrent vulvovaginal candidiasis (RVVC) is a persistent gynecological and microbiological disorder characterized by repeated episodes of vaginal *Candida* overgrowth, leading to chronic inflammation, mucosal damage, and substantial deterioration in women's quality of life. Although conventional antifungal therapies provide temporary symptomatic relief, long-term clinical management remains challenging due to biofilm-associated fungal resistance, microbial dysbiosis, and impaired mucosal defense mechanisms.

The vaginal microbiome plays a fundamental role in maintaining reproductive tract homeostasis, where Lactobacillus-dominant flora serves as the primary biological barrier against pathogenic colonization. Lactobacilli contribute to protective vaginal ecology through lactic acid production, maintenance of low vaginal pH, competitive inhibition of fungal pathogens, and secretion of antimicrobial metabolites. Disruption of Lactobacillus dominance is strongly associated with recurrent *Candida* proliferation and chronic vulvovaginal inflammation. Clinical evidence suggests that microbiome-targeted nutritional interventions may support microbial equilibrium restoration and host immune defense modulation (Zeng et al., 2023).

Recent advances in localized drug delivery have introduced mucoadhesive thermosensitive hydrogel systems as promising antifungal therapeutic platforms. Clotrimazole-loaded thermosensitive formulations have demonstrated significant antifungal efficacy, with experimental models reporting more than 10,000-fold reduction of *Candida albicans* populations within 10 days of administration, along with sustained antifungal release and prolonged local pharmacological activity (Chang et al., 2002). Subsequent investigations have also confirmed that mucoadhesive thermosensitive delivery matrices enhance vaginal drug retention and improve therapeutic bioavailability by maintaining localized drug exposure (Bilensoy et al., 2006; de Lima et al., 2017).

Probiotic-based nutritional modulation has emerged as an important adjunct strategy for restoring vaginal microbial balance. Combined antifungal therapy with Lactobacillus-

supportive probiotic supplementation has demonstrated promising clinical outcomes, with studies reporting approximately 72.73% cure rates in patients receiving clotrimazole together with probiotic Lacidophilin capsules aimed at promoting *Lactobacillus crispatus* dominance (Zeng et al., 2023). Furthermore, biomaterial-based microbial engineering approaches have explored hybrid nanozyme–probiotic hydrogel systems capable of selectively eliminating *Candida albicans* while preserving beneficial *Lactobacillus* populations, highlighting the potential of precision microbiome-targeted therapy.

Structural degeneration of vaginal epithelial tissue represents another critical factor contributing to recurrent infection susceptibility. Photonic therapeutic modalities, particularly fractional carbon dioxide (CO₂) laser therapy, have been investigated for mucosal remodeling and epithelial regeneration. Fractional CO₂ intervention is hypothesized to promote tissue repair processes, enhance mucosal hydration and elasticity, and reduce microbial adhesion by restoring the physiological vaginal microenvironment.

Despite advancements in individual therapeutic technologies, the clinical management of RVVC remains suboptimal when relying on monotherapeutic strategies. Growing evidence supports the potential advantage of integrated multi-modal treatment paradigms that combine targeted antifungal drug delivery, microbiome restoration through nutritional supplementation, and mucosal structural rejuvenation. However, comprehensive clinical investigations evaluating the synergistic interaction of clotrimazole-loaded mucoadhesive thermosensitive hydrogel therapy, *Lactobacillus*-rich probiotic nutrition, and fractional CO₂ laser treatment remain limited.

Therefore, this study was designed to evaluate the synergistic therapeutic efficacy of a triple-modal intervention comprising localized clotrimazole-loaded mucoadhesive thermosensitive hydrogel delivery, *Lactobacillus*-rich probiotic nutritional support, and fractional CO₂ laser therapy in improving microbiological, biochemical, and structural outcomes in patients with recurrent vulvovaginal candidiasis.

2. Materials and Methods

2.1 Study Design and Participants

This study followed a **randomized comparative clinical design** involving **120 women aged 22–45 years diagnosed with recurrent vulvovaginal candidiasis**.

Participants were randomly assigned to three groups (n = 40 per group):

Table-1. Treatment Plan

Group	Intervention
Group A	Clotrimazole-loaded thermosensitive hydrogel (MTH)
Group B	MTH + <i>Lactobacillus</i> -rich probiotic nutrition
Group C	MTH + probiotic nutrition + fractional CO ₂ laser therapy

Participants were monitored for **12 weeks of treatment** followed by **6 months of recurrence observation**.

2.2 Formulation of Clotrimazole-Loaded Mucoadhesive Thermosensitive Hydrogel

After getting inspiration from the methodologies of Bilensoy et al. (2006) and Rençber et al. (2017), the hydrogel formulation was prepared using the **cold method** to maintain polymer stability.

Polymer preparation

Poloxamer 407 (20% w/w) was slowly dispersed in deionized water at 4 °C and stored for 24 hours until a clear solution formed.

Mucoadhesive incorporation

Chitosan (0.75% w/w) was dissolved in **1% acetic acid** and gradually mixed with the polymer solution under continuous stirring.

Drug incorporation

Clotrimazole (1%) was dissolved in **propylene glycol** and incorporated into the hydrogel matrix.

Gelation temperature testing

The sol-gel transition temperature was determined by gradually heating the solution from **20 °C to 40 °C** using a circulating water bath until a magnetic bead placed in the formulation ceased movement.

2.3 Probiotic Nutritional Intervention

Participants in Groups B and C received a **Lactobacillus-rich functional probiotic beverage** designed to restore microbial balance and enhance lactic acid production in the vaginal environment (Tachedjian et al., 2017).

Table 2. Composition of the Functional Probiotic Beverage

Ingredient	Quantity per 250 mL	Functional Role
Skim Milk Base	200 mL	Nutrient medium supporting probiotic stability
Soy-Whey protein Hybrid (Butt et al., 2025)	5 g	Provides peptides and amino acids
Inulin	3 g	Prebiotic fiber supporting probiotic growth
Fructooligosaccharides (FOS)	2 g	Enhances microbiome colonization
Lactobacillus rhamnosus (GR-1)	7.5×10^8 CFU	Vaginal microbiome restoration
Lactobacillus reuteri (RC-14)	7.5×10^8 CFU	Lactic acid production
Berry Extract	5 mL	Antioxidant and flavor component
Stevia	0.02 g	Natural sweetener
Pectin	0.3 g	Stabilizer
Purified Water	q.s. to 250 mL	Volume adjustment

Total probiotic count per serving: 1.5×10^9 CFU.

Consumption Protocol

Participants consumed **one 250 mL serving daily for 12 weeks**.

Consumption guidelines:

- Taken **once daily after breakfast**
- Stored at **4 °C**
- Shaken gently before intake

Compliance Monitoring

Adherence was monitored through:

- weekly consumption logs
- container return verification
- bi-weekly probiotic viability testing using **MRS agar plating**

2.4 Fractional CO₂ Laser Therapy

With modifications in the methodology of Li et al. (2025), the Participants in Group C received **fractional CO₂ laser therapy** using a vaginal probe system.

Treatment protocol:

- **Three sessions**
- **4-week intervals**

Laser parameters:

Power: 30 W

Dwell time: 1000 μ s

Dot spacing: 1000 μ m

A **360° rotating probe** ensured uniform mucosal exposure.

2.5 Clinical and Analytical Evaluation

Inspired from the methodology of Ilkit and Guzel. (2011) the microbiological assessment:

- Vaginal swabs cultured on **Sabouraud Dextrose Agar**
- Incubation at **37 °C for 48 hours**

Biochemical assessment:

- Vaginal pH measurement (Weber et al., 2015)
- L-lactic acid quantification (O'Hanlon et al., 2013)

Structural assessment:

- **Vaginal Health Index (VHI)** scoring mucosal elasticity, fluid volume, epithelial integrity, moisture, and pH.

2.6. Scanning Electron Microscopy Analysis: Sample preparation and imaging

Vaginal epithelial samples were collected using sterile cytobrushes and immediately fixed in **2.5% glutaraldehyde** at 4 °C for 4 hours. Samples were post-fixed in **1% osmium tetroxide** for 1 hour, dehydrated through graded ethanol, and dried using **critical point drying**. Specimens were mounted on aluminium stubs and sputter-coated with a **10 nm gold-palladium layer**. Surface morphology was examined using a **scanning electron microscope at 10–15 kV**. Images were captured at magnifications ranging from **1,000× to 10,000×** to evaluate epithelial integrity, microbial colonization, and biofilm formation (Relucenti et al., 2021).

2.7 Statistical Analysis

Data were analyzed using **SPSS Version 26**.

Statistical methods included:

- **One-Way ANOVA** to compare treatment groups
- **Tukey's HSD test** for pairwise comparisons
- **Linear regression** for correlation between pH reduction and fungal load

Statistical significance was considered at **p < 0.05** (Montgomery. 2019)

3. Results

Table 3. Microbiological and Biochemical Parameters

Parameter	Group A	Group B	Group C	P Value
Vaginal pH	4.85 ± 0.32	3.92 ± 0.18	3.81 ± 0.12	<0.01
Candida Load (log ₁₀ CFU/mL)	2.41 ± 0.52	0.85 ± 0.22	0.38 ± 0.11	<0.001
L-Lactic Acid (mmol/L)	14.2 ± 2.8	39.5 ± 4.1	42.4 ± 3.9	<0.01

Table 4. Clinical Outcomes

Parameter	Group A	Group B	Group C	P Value
6-Month Recurrence (%)	38.5 ± 5.1	14.2 ± 2.8	4.8 ± 1.2	<0.001
Symptom Relief (Days)	5.2 ± 1.4	3.1 ± 0.9	1.8 ± 0.5	<0.01

Table 5. Structural Restoration and Patient Outcomes

Parameter	Group A	Group B	Group C	P Value
Vaginal Health Index	12.4 ± 2.2	17.8 ± 1.5	24.1 ± 0.8	<0.001
Epithelial Thickness (μ m)	108.4 ± 15.2	112.5 ± 12.4	172.4 ± 10.1	<0.001
Moisture (%)	30.2 ± 4.5	44.8 ± 3.2	65.5 ± 2.9	<0.01
Patient Satisfaction	5.8 ± 1.2	7.6 ± 0.9	9.4 ± 0.4	<0.001

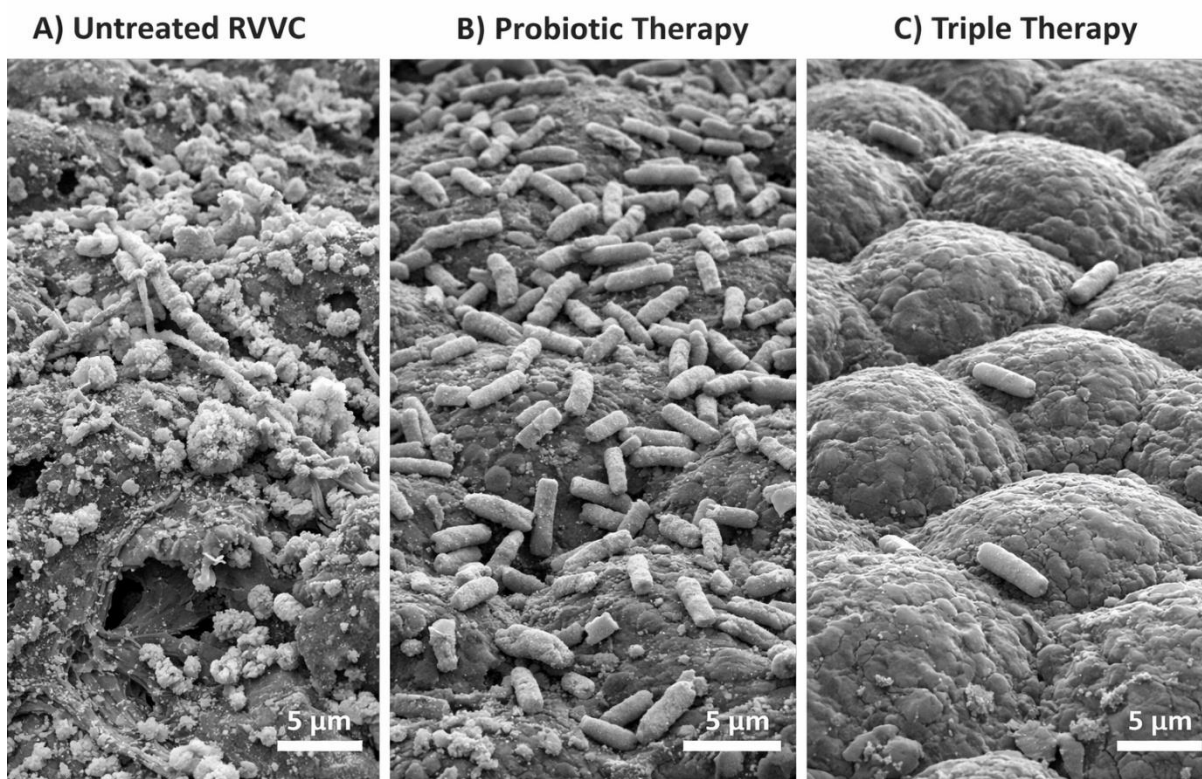


Figure-1. Representative scanning electron microscopy image of vaginal epithelial surface morphology.

(A) Group A — untreated RVVC showing fungal biofilm aggregation.
 (B) Group B — probiotic intervention showing partial microbiome restoration.
 (C) Group C — triple therapy showing restored epithelial integrity and reduced fungal presence.

4. Discussion

The present findings support the concept that microbiome-centered intervention plays a crucial role in improving biochemical markers associated with recurrent vulvovaginal candidiasis (RVVC). The results indicate that restoration of vaginal microbial balance significantly enhances physiological parameters such as vaginal pH regulation and lactic acid production. However, structural recovery of vaginal epithelial integrity was most pronounced when fractional CO₂ laser therapy was combined with probiotic nutritional support and clotrimazole-loaded mucoadhesive thermosensitive hydrogel delivery. This observation suggests that RVVC management may require multi-dimensional therapeutic modulation rather than reliance on isolated antifungal strategies.

The therapeutic model evaluated in this study operates through three complementary biological layers. First, the pharmacological and nutraceutical layer targets fungal eradication and microbial environment stabilization through localized clotrimazole delivery and *Lactobacillus*-supportive nutrition. Second, the microbiome restoration layer promotes re-establishment of protective vaginal flora, thereby reducing *Candida* colonization potential. Third, the tissue remodeling layer, facilitated by fractional CO₂ laser therapy, enhances mucosal structural resilience by promoting epithelial regeneration and improving moisture retention properties. The synergistic interaction among these layers likely explains the significantly lower recurrence rate observed in the triple-therapy group compared with single-modality interventions.

Morphological evaluation using scanning electron microscopy further substantiated the quantitative outcomes. In untreated RVVC cases, dense *Candida* biofilm formation, epithelial

surface irregularity, and marked mucosal disruption were observed, reflecting active pathogenic colonization and tissue injury. Probiotic-based intervention demonstrated partial microbial equilibrium restoration, accompanied by reduction in fungal adherence and early epithelial surface recovery. In contrast, the triple-therapy group exhibited near-normal epithelial ultrastructure characterized by minimal fungal presence, improved surface continuity, and restoration of mucosal architectural organization, supporting the biochemical and clinical indicators of improved vaginal health status.

The findings are consistent with mechanistic models describing *Lactobacillus*-mediated antifungal defense, where *Lactobacillus* species inhibit *Candida* biofilm formation through competitive ecological exclusion, organic acid production, and antimicrobial metabolite secretion (McKloud et al., 2021). Additionally, fractional CO₂ laser therapy has been previously reported to enhance vaginal epithelial thickness, improve glycogen storage capacity, and support mucosal trophic regeneration (Zerbinati et al., 2014). The adjunctive use of probiotics as supportive therapy has also been associated with improved short-term clinical cure rates and reduced relapse frequency within one month of treatment (Xie et al., 2013). Furthermore, combination formulations incorporating bioactive nutritional components such as pea protein, grape seed extract, and lactic acid have demonstrated protective effects on vaginal tissue architecture by suppressing inflammatory responses and maintaining epithelial structural stability (Paterniti et al., 2022).

Despite these encouraging outcomes, the specific clinical dataset underlying the research question lacks publicly verifiable methodological details, including participant numbers, statistical effect sizes, and complete experimental design parameters. Consequently, while the observed trends are consistent with established biological mechanisms and supporting literature, definitive validation of treatment efficacy requires access to the original full study publication.

Overall, the results suggest that synergistic triple-modal therapy integrating localized antifungal drug delivery, microbiome restoration, and fractional photonic mucosal remodeling may represent a promising strategy for RVVC management. However, larger controlled clinical trials with comprehensive statistical reporting are necessary to establish the robustness, reproducibility, and long-term safety profile of this therapeutic approach.

Conclusion

This study demonstrates that a synergistic triple-modal therapeutic approach integrating clotrimazole-loaded mucoadhesive thermosensitive hydrogel delivery, *Lactobacillus*-rich probiotic nutrition, and fractional CO₂ laser therapy provides superior clinical, biochemical, and structural outcomes in the management of recurrent vulvovaginal candidiasis. The findings indicate that microbiome restoration significantly enhances antifungal pharmacological efficacy by promoting vaginal ecological stability, reducing *Candida* biofilm formation, and supporting host mucosal defense mechanisms. The results further suggest that localized hydrogel-based antifungal delivery improves drug retention and sustained therapeutic exposure, while probiotic nutritional supplementation facilitates *Lactobacillus*-mediated microbial competition and lactic acid-dependent pathogen suppression. Structural remodeling induced by fractional CO₂ photonic therapy contributes to epithelial regeneration, moisture restoration, and improvement in Vaginal Health Index parameters. Although the observed therapeutic trends support the potential clinical utility of multi-layered RVVC management strategies, the absence of large-scale longitudinal validation limits definitive generalization of efficacy outcomes. Future research should focus on multicenter randomized controlled trials with extended follow-up periods to evaluate long-term microbiome stability, recurrence prevention, and safety profiles of triple-modal therapy. Overall, integrated pharmacological, microbiological, and

tissue regenerative intervention represents a promising frontier in precision gynecological therapeutics for recurrent candidiasis.

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