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Lactobacillus plantarum in high methoxylated pectin-based encapsulation systems supports gut epithelial wound healing *in vitro*

Felipe Galvez-Jiron [a,b,c,*], Lucas de Freitas Pedrosa [a,d], Luis Silva Lagos [a], Xin Tang [a], Traudy Wandersleben [e], Rodrigo Navia [c,f], Stephan Drusch [g], Francisca Acevedo [c,h,i], Paul de Vos [a,j]

[a] *Immunoendocrinology, Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*
[b] *Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada, Facultad de Medicina, Universidad de La Frontera, Temuco, 4811230, Chile*
[c] *Millennium Nucleus Bioproducts, Genomics and Environmental Microbiology (BioGEM), Avenida España 1680, 2390123, Valparaíso, Chile*
[d] *Department of Food Science and Experimental Nutrition, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil*
[e] *Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Avenida Francisco Salazar, 01145, Temuco, Chile*
[f] *Facultad de Ingeniería, Universidad San Sebastián, Bellavista 7, Santiago, Chile & Centre for Biotechnology and Bioengineering (CeBiB), AFB240001, Chile*
[g] *Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Department of Food Technology and Food Material Science, Königin-Luise-Str. 22, 14195, Berlin, Germany*
[h] *Center of Excellence in Traslational Medicine (CEMT), Faculty of Medicine, and Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Casilla 54-D, Temuco, Chile*
[i] *Department of Basic Sciences, Faculty of Medicine, Universidad de La Frontera, Casilla 54-D, Temuco, Chile*
[j] *Centre for Healthy Eating & Food Innovation (HEFI), Maastricht University - campus Venlo, 5928, SZ, Venlo, the Netherlands*

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ABSTRACT

Wound healing and gut epithelial barrier regulation are crucial for intestinal homeostasis. Effective repair of gut epithelial wounds is crucial for re-establishing the mucosal barrier and resolving inflammation. Pectins, versatile polysaccharides in food, protect sensitive components, such as living bacteria, during gastrointestinal transit and support wound healing by promoting cell proliferation and migration.

This study investigated the effects of encapsulated *Lactobacillus plantarum WCFS1* on cell proliferation and migration during epithelial wound healing, using pectin-based beads stabilized with alginate or chitosan. Pectin from lemon, with two degrees of methyl esterification (DM18 and DM88), produced four microbead types. T84 cells were incubated with these beads for 24 hours. The results revealed that cell proliferation and migration were influenced by the bead type, the degree of methyl esterification of the pectin, and the inclusion of *L. plantarum*. Empty DM88 pectin beads improved cell proliferation while reducing migration tendencies, whereas DM18 beads delayed migration.Beads containing *L. plantarum* demonstrated different effects: DM18 beads enhanced proliferation without inhibiting migration, while DM88 beads reduced migration. Both empty and *L. plantarum*-loaded beads decreased CCL20 production. Alginate/pectin beads with *L. plantarum* increased IL-8, while chitosan/pectin beads delayed migration, reduced CCL20 secretion, and modulated IL-8 levels.

In conclusion, DM88 pectin beads without *L. plantarum* and DM18 beads containing *L. plantarum* supported cell proliferation over migration during epithelial wound healing. These findings suggest that pectin-based encapsulation systems may play a role in promoting gut health, protecting barrier integrity, and delivering therapeutic agents.

* Corresponding author at: Hanzeplein 1, EA11, 9713 GZ, Groningen, the Netherlands.
E-mail addresses: f.e.galvez.jiron@umcg.nl, f.galvez01@ufromail.cl (F. Galvez-Jiron), lfpedrosa@usp.br (L.F. Pedrosa), l.a.silva.lagos@umcg.nl (L.S. Lagos), x.tang@umcg.nl (X. Tang), traudy.wandersleben@ufrontera.cl (T. Wandersleben), rodrigo.navia@uss.cl (R. Navia), stephan.drusch@tu-berlin.de (S. Drusch), francisca.acevedo@ufrontera.cl (F. Acevedo), p.de.vos@umcg.nl (P. de Vos).

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1. Introduction

The intestinal epithelial cells form the boundary between the internal and external environment of the intestine and are the first physical barrier against foreign pathogens (Okamoto & Watanabe, 2016). In homeostasis, they form a selective permeability barrier separating luminal content from underlying tissues (Iizuka & Konno, 2011). This intestinal epithelial homeostasis is maintained by balancing the rate between cell proliferation and cell loss in which apoptosis accounts for most cell loss in the gut lumen (Ramachandran et al., 2000) and is based on a delicate balance maintained by multiple components, which is constantly self-renewed. At the same time, alterations in the mucosal structure are a major feature of many inflammatory gut issues such as in inflammatory bowel disease but also in other gut injuries (Ahmad et al., 2017; Villablanca et al., 2022). Injuries to the intestine typically involve several changes, including abnormalities in epithelial cells, reduced morphology and density of colonic crypts, alterations in lamina propria cells, and immune cell infiltration (Kobayashi et al., 2020). To maintain tissue homeostasis under altered pathophysiological conditions, prompt compensatory changes in cell proliferation, migration, and cell death are essential. These processes are critical for preserving organ function and preventing disease (Bao et al., 2020).

Upon injury, the intestinal epithelium undergoes a wound healing process. This intestinal wound healing depends on the balance of three cellular events; restitution, proliferation, and differentiation of epithelial cells adjacent to the wounded area (Moyer et al.2007). Previous studies have shown that various regulatory peptides, including growth factors and cytokines, modulate intestinal epithelial wound healing (Sturm & Dignass, 2008). Epithelial cells surrounding the wound lose their columnar polarity, take on a flattened morphology, and rapidly migrate into the denuded area to restore barrier integrity (Dignass & Podolsky, 1993; Dise et al., 2008; Sturm & Dignass, 2008). Restitution starts within minutes to hours of injury and is independent of proliferation (Dignass & Podolsky, 1993; Sturm & Dignass, 2008). The proliferation of the mucosal epithelium, which increases the number of enterocytes needed to repair the damaged area, typically begins within hours to days after the injury (Dignass & Podolsky, 1993). Finally, maturation and differentiation of epithelial cells are needed to maintain the mucosal barrier function (Sturm & Dignass, 2008).

Strategies to support or prevent wound healing in the gut have been studied to a great extent (Sommer et al., 2021). One of these approaches is by using specific types of probiotics (Bădăluță et al., 2024). Various studies show the potential of probiotics in promoting wound healing by inhibiting pathogen attachment and competing for adherence sites, nutrients, and other vital resources (Barzegar et al., 2023). Additionally, some probiotics protect the intestinal epithelium against inflammation and enhance epithelial barrier function (Cristofori et al., 2021; Li et al., 2023). Probiotics also promote cell survival, stimulate the production of antibacterial substances and cell-protective proteins, support protective immune responses, and suppress the production of proinflammatory cytokines (Yan & Polk, 2020;Masheghati et al., 2024). One of the potential wound healing supporting beneficial bacteria is *Lactobacillus plantarum*. This strain can enhance intestinal healing by reducing mucosal inflammation and promoting intestinal mucosal growth (Liu et al., 2001), further benefits including prolonging food shelf-life, enhancing antioxidant activity, improving food flavor characteristics and antimicrobial activities in the food industry, and application as a potential starter for dairy products have been attributed to it (Zare et al., 2024). This strain also has been reported to stimulate the proliferation and differentiation of intestinal epithelial stem cells *in vitro*, by accelerating epithelial proliferation and differentiation (Hou et al., 2018). However, many beneficial bacteria struggle to survive the variations in pH and the harsh enzymatic conditions of the gastrointestinal tract. For probiotics to be effective in preventing or supporting gut healing, they need to reach the colon and successfully colonize the mucosa. To address this, microencapsulation in biodegradable prebiotics has been proposed as a protective method. This approach enhances bacterial viability during processing and ensures their delivery to the distal areas of the intestine (Peñalva et al., 2023).

A possible encapsulation approach for probiotic species may involve pectin-based microcapsules. Pectins are indigestible carbohydrates selectively fermented by intestinal bacteria that promote the development and activity of beneficial microbes. Pectins are nowadays preferred for encapsulation as they have health benefits as such. Depending on their methyl esterification grade (DM) they can lower inflammation by binding to specific immune receptors (Beukema et al., 2021; Morales-Medina et al., 2022) and they can support the production of short-chain fatty acids (SCFA) by gut microbiota that also have beneficial effects (Elshahed et al., 2021). Whether they also have effects on gut wound healing processes remains to be determined (Kowalonek, 2017; Morales-Medina et al., 2022). As a biopolymer, pectin allows for encapsulation under mild conditions, supporting the metabolism and survival of enclosed cells. Pectins are resistant to the acidic pH of the stomach and digestive enzymes, reaching the intestine in an intact form, where they are fermented by microbiota and releasing their cargo. These properties not only protect chemically unstable compounds (Rosales & Fabi, 2023) but also facilitate the colonization of encapsulated probiotics due to their prebiotic nature (Sun et al., 2024). Structurally, pectin is an anionic polysaccharide that interacts with divalent cations, such as calcium ions, through its non-methyl-esterified galacturonic acid units to form gel networks. This "egg-box" gelation mechanism stabilizes the homogalacturonan chains. However, calcium-pectin gels often exhibit mechanical weaknesses and shape deformation (Michon et al., 2004; Celus et al., 2018; Said et al., 2023). To address these issues, stabilizing agents such as alginate or chitosan are sometimes added to improve the mechanical properties of pectin beads. Both pectin and alginate are natural anionic polysaccharides that form hydrogels through chain–chain associations when divalent cations, such as Ca^{2+}, are present. Chitosan, on the other hand, is a weak polybase that interacts electrostatically with the weakly acidic pectin. The positively charged NH_3^+ groups in chitosan bind with the negatively charged carboxyl groups ($-COO^-$) in pectin to form stable complexes (Fang et al., 2008; Jindal et al., 2013; Kowalonek, 2017; Rezvanian et al., 2017). These interactions enhance the strength and stability of pectin-based microcapsules, making them an effective delivery system for probiotics.

Recents studies support the alternative use of biopolymer hydrogels and probiotic encapsulation strategies to improve wound healing. Pectin-chitosan hydrogels loaded with ciprofloxacin for antibacterial and healing wound applications was tested. *In vitro* and in *vivo* pharmacodynamics experiments have shown that the hydrogels can resist bacteria and promote wound healing (Song et al., 2023). Hydrogel wound dressings were developed using photocrosslinked pectin and gelatin. These dressings were designed to release curcumin, a natural antimicrobial agent, in a pH-dependent manner. The curcumin-loaded hydrogels demonstrated good cytocompatibility with fibroblast cells and exhibited antibacterial activity against *Staphylococcus aureus*. (Bostancı et al., 2022). A multifunctional hydrogel wound dressing was developed using low methoxyl pectin to enable sustained release of procaine. This dressing demonstrated strong hemostatic and antioxidant properties. *In vivo* data from both a mouse skin incision model and an infected full-thickness skin wound model revealed a notably high rate of wound closure. These findings underscore the hydrogel's effectiveness in promoting wound healing and highlight its considerable potential in advanced wound care management (Kocaağa et al., 2024). A probiotic-in-hydrogel wound dressing platform was developed using *Lactobacillus casei* as the model probiotic. This strain was encapsulated within an alginate-based system consisting of macrogels, microgels, and biofilm structures. The resulting formulation exhibited notable antibacterial activity and biological compatibility. In a diabetic mouse wound model, the dressing further demonstrated significant pro-healing effects, including enhanced vasculogenesis and reduced inflammation (Xin et al., 2025).

This study aims to evaluate the impact of *L. plantarum WCFS1* in pectin-based beads on intestinal wound healing processes. To this end, we used pectins with either 18 and 88 DM and combined this with alginate or chitosan as stabilizing agents. First, the impact of the capsules as such was studied on wound healing processes followed by studies on *L. plantarum*-loaded pectin-based beads. The effects were tested on T84-gut epithelial cell monolayers, using a wound healing *in vitro* assay in the absence and presence of mitomycin C to evaluate cell proliferation and migration. Supernatants were collected to measure the release of IL-6, IL-8, CCL20, IL-13, IL-28a, and IL-33 using a magnetic Luminex Assay. This study is the first to evaluate the effects of pectin-based beads on cell proliferation and migration.

2. Materials and methods

2.1. Materials

Cell culture material was purchased from BD Falcon (BD Biosciences, USA). Calcium chloride dihydrate ($CaCl_2 \times 2H_2O$), glacial acetic acid, and ammonia solution 32% were purchased from Merck (Germany). Chitosan from the fungus *Agaricus bisporus* was acquired from KiOsmetine®CsH (CsH) and had a degree of acetylation below 20%, and an average molecular weight of 60kDa. High guluronic acid (G) alginate (>50% G) was purchased from ISP Alginates (Manugel® DMB, Tadworth, UK). Mitomycin C was acquired from Sigma Aldrich (UK). The extracted lemon pectin with a degree of methyl-esterification DM18 (LPDM18) and DM88 (LPDM88) was purchased from CPKelco (Denmark), and the composition of the pectin samples is shown in Table 1. The degree of blockiness was calculated as previously described (Beukema et al., 2021).

2.2. Bacterial strain, culture condition

Lactobacillus plantarum WCFS1 was maintained at -80°C in De Man-Rogosa-Sharpe (MRS) broth and 20% of sterile glycerol and was spread in MRS agar and cultured at 37°C for 24 h under aerobic conditions. Before the experiments, the bacteria were sub-cultured in 10 mL MRS broth and incubated at 37°C for 24 h. The bacterial suspension was diluted 10 times into MRS broth, and the concentration was adjusted to 0.5 OD600 (approximately 10^7CFU/mL). The bacteria were centrifuged at 10,000 g at 4°C for 10 min, the supernatant was discarded, and the pellet was suspended in 0.8 mL of both LPDM18 1.5% and LPDM88 2.7% solution.

2.3. Bead formation using electrostatic droplet generation

2.3.1. Alginate/lemon pectin beads production

Alginate/lemon pectin bead systems were produced using the same electrodripping system (Bugarski et al., 1994). The powdered polymers were previously sterilized under UV light overnight, before use. Both DM18 lemon pectin (LPDM18) (2.0% w/v) and DM88 lemon pectin (LPDM88) (2.0% w/v) solutions were mixed with alginate solution (1.9 % w/v) in a ratio of 1:3. The solution was extruded as described above with a few modifications. The distance between the solution's surface and the needle was 7.5 cm. Both alginate/pectin DM18 (AlgDM18) and alginate/pectin DM88 (AlgDM88) empty beads were also stored in 100 mM $CaCl_2 \times 2H_2O$, 2 mM KCl, 10 mM Hepes. As described above, the alginate/lemon pectin beads loaded with *L. plantarum* were produced from *L. plantarum* with 0.8 mL of either AlgDM18 or AlgDM88 solution. Both bead types, i.e., AlgDM18 and AlgDM88 with *L. plantarum WCFS1*, were produced as described above and stored in Krebs-Ringer solution (HEPES buffered, 1mM D-glucose) until further use.

2.3.2. Chitosan/lemon pectin beads production

Chitosan/lemon pectin bead systems were produced using an electrodripping system (Bugarski et al., 1994). The powdered polymers were previously sterilized under UV light overnight, before use. Both LPDM18 (1.5% w/v) and LPDM88 (2.7% w/v) plus ammonia solution (2.0% v/v, pH 10), solution were extruded using a syringe pump PHD 2000 infusion (Harvard Apparatus, USA) through a needle connected to an electrostatic accelerator (Nisco Engineering, Switzerland). This generator was placed above a beaker containing the chitosan solutions (2.7% w/v in 130 mM $CaCl_2 \times 2H_2O$, both acetic acid 0.5% v/v or 2.5% v/v). The needle (25G inner size 260 μm, outer size 530 μm) was fixed into the electrodripping device through luer lock connectors. The distance between the solution's surface and the needle was 12.5 cm. The potential difference was controlled with a voltage power supply with a variable voltage of 10 kV. Both pectin aqueous solutions were extruded with a syringe (5 mL capacity) and pump (flow rate set at 7.5 mL/min) into a 50 mL chitosan solution. Chitosan/lemon pectin beads were formed upon contact with the bath solution and were left to harden for 30 min. After hardening, the beads were removed from the solution by filtration. ChitoDM18 and ChitoDM88 empty beads were stored in a 100 mM $CaCl_2 \times 2H_2O$, 2 mM KCl, 10 mM Hepes solution. As described above, the chitosan/lemon pectin beads loaded with *L. plantarum* were produced from *L. plantarum* with 0.8 mL of either LPDM18 or LPDM88 solution. Both bead types, i.e., ChitoDM18 and ChitoDM88 with *L. plantarum* WCFS1, were made as described above and stored in Krebs-Ringer solution (HEPES buffered, 1mM D-glucose) until further use (Galvez-Jiron et al., 2025).

2.4. Cell culture

T84 human epithelial colonic cells (ATCC, USA) were used between passages 14 and 22. T84 cells were cultured in Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham with 15 mM HEPES, 2.5 mM L-glutamine (Gibco, Thermo Fisher Scientific), 50 mg/mL gentamicin (Lonza, Belgium), 10% heat-deactivated fetal bovine serum (Merck, Germany). T84 cells were cultured at 37°C under 5% CO_2 in humified air. The medium was refreshed every other day until 80% confluence was reached. For maintenance, the cells were passaged after treatment with 0.1% trypsin-EDTA (Sigma-Aldrich, Dorset, UK). T84 cells were cultured for 14 days until confluence was reached and used in the experiments.

2.5. Wound healing assay

Epithelial T84 cells were seeded at a density of 10,000 cells per well in a final volume of 300 μL into a 96-well plate. The confluent T84 cell monolayers were pre-incubated for 24 hours with 6, 8, or 10 beads of either alginate or chitosan/lemon pectin beads, which were previously considered and reported to test protective role in intestinal barrier disruption model (Galvez-Jiron et al., 2025). Afterward, the beads were removed and the cell monolayers were washed twice with PBS and

Table 1
Structural characteristics of pectins.

				Sugar composition (mol%)					
	Origin	DB (%)	M_w (kDa)	Rha	Ara	Gal	Glc	UA	Carbohydrate content (w/w%)
DM18	Lemon	86	78	1	0	2	0	97	62
DM88	Lemon	91	91	1	3	5	0	97	67

refilled with fresh media. Then the monolayer was treated with 5 μg/mL of Mitomycin C (Sigma-Aldrich, UK) or vehicle control (distillate water) for 2 hours. Next, for the wound healing assays, confluent cells were scratched using the Incucyte 96-well Wound maker device (Sartorius, United Kingdom) and washed twice to remove floating cells and prevent reattachment in the wound area. The cells were imaged in phase contrast using the 10x objective in Incucyte S3 Live Cell Analysis System with Incucyte S3 software (Sartorius). Images were taken every 2 hours for a period of 48 hours.

2.6. Cell migration analysis

Cell tracking was performed manually using the ImageJ Manual Tracking plugin to calculate migration parameters (Suarez-Arnedo et al., 2020). Images at baseline were taken immediately after creating the open wound area. IncuCyte ZOOM™ (10X) images were analyzed for wound closure of the scratch using Fiji ImageJ (Schindelin et al., 2012). The scratch area, wound coverage of the total area, and average and standard deviation of the scratch width were determined. The percentage of wound closure was calculated according to according to (Eq 1) (Grada et al., 2017):

$$Wound\ Closure\ \% = \frac{At = 0h - At = \Delta th}{At = 0h}\ x\ 100\%$$

Where $At = 0$ is the initial wound area, $At = \Delta t$ is the wound area after n hours of the initial scratch.

2.7. Assessment of cytokine production

A magnetic Luminex Assay (R&D systems, Bio-Techne, USA) was used to measure the production of C-X-C motif ligand 8 (CXCL8)/IL-8, monocyte chemoattractant protein-3 (MCP-3)/C-C chemokine ligand 20 (CCL20), Interleukin (IL)-6, IL-13, IL-28a and IL-33 by the gut epithelial cells. To this end, cell supernatants were collected when the wound healing assay was finished. The assay was performed according to the manufacturer's protocol. Briefly, cytokine standards were resuspended, and serial dilutions were prepared. Antibody magnetic bead mixes were added to a 96-well plate. Standards and samples were added and incubated overnight at 4°C while shaking. After washing the plate three times, detection antibodies were added, and the plate was incubated for 30 min at RT while shaking. After incubation, the plate was washed and incubated with streptavidin-PE for 30 min at RT while shaking. Finally, the plate was washed again, and 100 μL of wash buffer was added to each well. Subsequently, the plate was analyzed using a Luminex 200 System. The data obtained was analyzed using the Luminex xPONENT software.

2.8. Statistical analysis

The GraphPad Prism software version 10.0.0 was used for statistical analyses. Normal distribution was confirmed using the Kolmogorov-Smirnov test. The results that were normally distributed were expressed as mean ± standard error of mean (SEM). One-way ANOVA was used for statistical comparisons, and post-testing was performed with Tukey's test to test statistical differences between groups ($^*p < 0.05$ was considered statistically significant; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). * Represent the groups were compared with control and ■ represent groups were compared with MMC untreated and treated for statistical comparisons. Each sample was tested 5 times (triplicates) and the mean of each sample was used for statistical comparisons.

3. Results

To assess the impact of pectin-based beads on intestinal epithelial cell proliferation and migration, we employed an *in vitro* wound closure assay using confluent T84 colonic epithelial cell monolayers. Wound closure reflects both cell proliferation and migration. To distinguish between these processes, we included mitomycin C (MMC), a proliferation inhibitor that blocks DNA synthesis (Tomasz, 1995), thereby isolating migration effects. At 12, 24, and 48 hours post-wounding, proliferating cells covered 23.2 ± 0.7%, 26.1 ± 0.9%, and 33.4 ± 2.7% of the wound width, respectively, while MMC-treated migrating cells covered 18.8 ± 1.4%, 21.5 ± 1.2%, and 26.7 ± 0.5% (**Fig. 1a; Fig. S1 a–f**). The characteristics of the pectin-based beads were previously described (Galvez-Jiron et al., 2025). We evaluated both empty and *L. plantarum*-loaded alginate-pectin beads, followed by chitosan-pectin variants, examining the influence of pectin's degree of methylation (DM) on wound healing performance in each system.

3.1. Empty alginate-DM88 pectin beads support proliferation while the other bead types had no effect

First, the impact of empty alginate-pectin-based beads pre-treatment on the proliferation and migration of wound inflicted gut T84 epithelial cells were tested. The confluent T84 cell monolayers were pre-incubated for 24 hours with 6, 8, or 10 alginate-pectin-based beads. Afterward, the beads were removed and the cell monolayers were washed. Then the monolayer was treated with vehicle control (non-MMC) or 5 μg/mL of Mitomycin C (MMC) for 2 hours before applying the scratch.

The pre-treatment with either number of empty beads did not increase the proliferation and the migration of T84 cell in the monolayers at 12, 24 and 48 hours (Fig. 1**a, b, c, d,**) with one exception. The pre-treatment with AlgDM18 10c empty beads did produce a tendency to increase cell proliferation to 30.9 ± 3.4% at 12 hours, 31.9 ±3.5% at 24 hours and 39.8 ± 2.5% at 48 hours in non-MMC monolayers. The migration of MMC T84 cells over 48 hours did not improve by pre-treatment of either amount of empty AlgDM18 empty beads, but when the untreated and pre-treated mitomycin C cells were compared between the same amount of beads, AlgDM18 10c empty beads showed differences at 12 hours ($p \leq 0.0001$), 24 hours ($p \leq 0.01$) and 48 hours ($p \leq 0.0001$) (Fig. 1**a, b, c, d, Fig. S2).** This suggests that AlgDM18 at a higher bead density of 10c improves cell proliferation rather than migration. On the other hand, T84 cell monolayer pre-treated with AlgDM88 10c increased cell proliferation to 31.1 ± 3.1% ($p<0.05$) at 12 hours, 34.9 ± 2.1% ($p<0.05$) at 24 hours, and 45.9 ±2.8% ($p \leq 0.01$) in non-MMC T84 cell monolayer. However, the pre-treatment of AlgDM88 6c reduced proliferation in non-MMC cell monolayer to 12.3 ± 1.9% ($p<0.01$) at 12 hours, 14.6 ± 1.4% ($p<0.0001$) at 24 hours, but no differences were found at 48 hours (Fig. 1**e, f, g, h**). AlgDM88 10c induce a decrease in cell migration to 10.5 ± 1.6% ($p<0.01$) at 24 hours and 12.5 ± 2.1% ($p<0.01$) at 48 hours in MMC T84 cell monolayers (Fig. 1**e, f, g, h, Fig. S3**). Differences were found at AlgDM88 8c and 10c when they were compared between non-treated and pre-treated mitomycin C cell monolayers at 12, 24 and 48 hours ($p<0.0001$), suggesting that AlgDM88 beads could enhance cell proliferation rather than migration (Fig. 1**e, f, g, h**).

3.2. Alginate-pectin-based empty beads decrease the production of CCL20

Next, we investigated whether the beads influenced epithelial immune responses. To do this, we quantified the levels of CCL20, IL-6, IL-8, IL-13, IL-28a, and IL-33 in the supernatant of T84 cell monolayers 48 hours after scratching. These cytokines, measured in both non-MMC and MMC conditions, play a critical role in regulating intestinal epithelial proliferation, cell migration, and barrier permeability (Okumura & Takeda, 2017; Xue & Falcon, 2019). IL-6, IL-13, IL28a, and IL-33 were not detectable in the supernatant but CCL20 and IL-8 were regulated by the alginate-pectin-based empty beads. The control of non-MMC and MMC T84 cell monolayer produced 3658 ± 109 pg/mL and 250.9 ± 59.7 pg/mL of CCL20, and 1304 ± 154 pg/mL and 618.3 ± 131.7 pg/mL of IL-8.

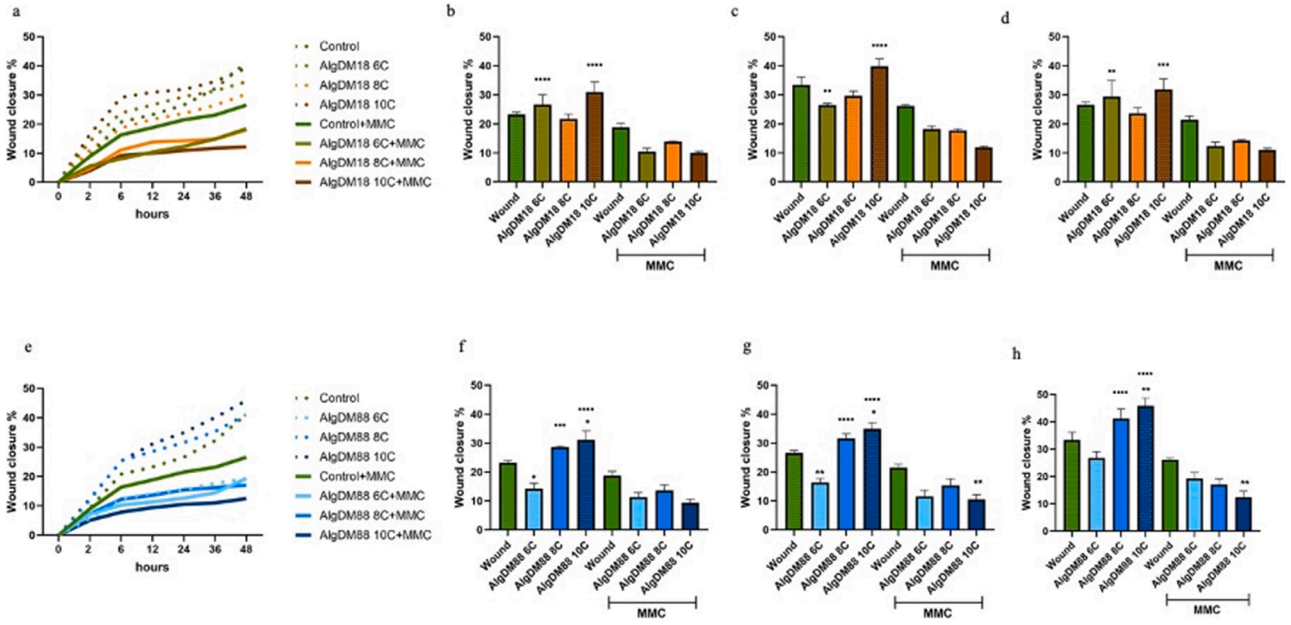

Fig. 1. The effect of empty alginate-pectin-based beads on cell proliferation and cell migration. **a**) Wound closure was measured over 48 hours in non-MMC and MMC T84 cell monolayer after the pre-treatment of AlgDM18 6, 8, and 10 beads. Wound closure in non-MMC and MMC T84 cell monolayers after the pre-treatment of AlgDM18 at 12 (**b**), 24 (**c**) and 48 hours (**d**) after the scratch assay. **e**) Wound closure was measured over 48 hours on untreated and pre-treated mitomycin T84 cell monolayer after the pre-treatment of AlgDM88 6, 8, and 10 beads. Wound closure in non-MMC and MMC T84 cell monolayers after the pre-treatment of AlgDM88 at 12 (**f**), 24 (**g**) and 48 hours (**h**) after the scratch assay. Data are expressed as mean ± SEM. Groups were compared with wound control and presented as %. N=5 independent experiments with two technical replicates for each condition Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

For CCL20, a significant reduction was observed in non-MMC T84 cell monolayers pre-treated with either concentration of AlgDM18 or AlgDM88 empty beads. After 48 hours post-scratching, CCL20 production decreased markedly ($p<0.0001$). Treatment with AlgDM18 empty beads resulted in approximately a tenfold reduction in CCL20 levels, regardless of the concentration. In contrast, AlgDM88 empty beads reduced CCL20 secretion to a lesser extent, with an approximate fivefold decrease compared to the control (Fig. 2**a**). No differences were found in the production of CCL20 in MMC T84 cell monolayers pre-treated with either amount of both AlgDM18 or AlgDM88 beads (Fig. 2**c**). For IL-8, secretion was not significantly affected by pre-treatment with either concentration of AlgDM18 or AlgDM88 empty beads in both non-MMC and MMC T84 cell monolayers (Fig. 2**b**). However, pre-treatment with 8c AlgDM18 empty beads in MMC T84 cell monolayers showed a tendency to double IL-8 secretion compared to the control. (Fig. 2**b**).

3.3. *L. plantarum WCFS1-loaded alginate-DM18 pectin beads improve cell proliferation than cell migration*

After studying the impact of empty alginate-pectin-based beads pre-treatment as such on proliferation and migration in the wound healing assay we determined how the effects of *L. plantarum WCFS1* on wound healing was maintained by the alginate-pectin-based beads. AlgDM18 and AlgDM88 were loaded with *L. plantarum* WCFS1, then 6, 8, and 10 of these beads were tested in non-MMC and MMC T84 cell monolayers as described above.

Different effects were found by alginate-pectin-based beads loaded with *L. plantarum WCFS1* on cell proliferation and migration. *L. plantarum WCFS1* in AlgDM18 had a significant impact on cell proliferation and a tendency to lower migration. On the other hand, *L. plantarum WCFS1* in AlgDM88 tended to decrease cell proliferation and reduced cell migration.

The pretreatment with *L. plantarum WCFS1* in AlgDM18 6c did increase the proliferation in non-MMC T84 cell monolayer 35.3 ±1.8% ($p<0.05$) at 24 hours and 45.6 ±2.4% ($p<0.01$) at 48 hours (Fig. 3**a, b, c, d**). No significant differences were observed at 12 hours or with AlgDM18 8c and 10c pre-treatment. On the other hand, the pre-treatment with AlgDM18-Lp 10c decreased the migration of MMC T84 cell monolayer to 10.9 ±1.8% ($p<0.05$) at 24 hours and to 13.15 ±1.8% ($p<0.001$) at 48 hours (Fig. 3**a, b, c, d, Fig. S4**). When the different amounts of AlgDM18-Lp were compared between untreated and pre-treated mitomycin C cell monolayers, AlgDM18-Lp 6c showed higher differences at 12, 24 and 48 hours, and 8c and 10c showed differences at 48 hours after scratching (Fig. 3**a, b, c, d**).

The proliferation of non-MMC T84 cell monolayer pre-treated with *L. plantarum WCFS1* in AlgDM88 at 6c, 8c and 10c was reduced to 18.5 ± 2.4% ($p<0.001$), 21.3 ± 1.1% ($p<0.01$), and 23.3 ± 3.0% ($p<0.05$) at 12 hours, respectively. This reduction was maintained until 24 hours with AlgDM88-Lp 6c to 19.5 ± 1.8% ($p<0.05$) (Fig. 3**e, f, g, h**) Cell migration was also affected in the MMC T84 cell monolayer. Pre-treatment with AlgDM88-Lp showed slower migration compared to the control. Specifically, *L. plantarum WCFS1* encapsulated in AlgDM88 at a concentration of 6c reduced cell migration to 10.3 ± 2.7% ($p<0.0001$), 11.1 ± 2.6% ($p<0.01$), and 14.5 ± 2.3% ($p<0.01$) at 12, 24 and 48 hours post-scratching, respectively. Similarly, pre-treatment with *L. plantarum* WCFS1 in AlgDM88 at a concentration of 8c decreased cell migration at 12 hours to 11.5% ($p<0.001$) and at 24 hours to 12.1% ($p<0.01$) after the scratch (Fig. 3**e, f, g, h, Fig. S5**).

3.4. *L. plantarum WCFS1-loaded alginate-pectin-based beads reduce the secretion of CCL20 on cell proliferation and increase the production of IL-8 on cell migration*

Next, we determined whether *L. plantarum WCFS1* in alginate-pectin-based beads had an effect on the epithelial immune responses by quantifying CCL20, IL-6, IL-8, IL-13, IL28a, and IL-33 in the supernatant of non-MMC and MMC T84 cell monolayer after 48 hours of scratching as described above.

L. plantarum WCFS1 in alginate-pectin-based beads did reduce the secretion of CCL20 on non-MMCT T84 cell monolayer. However,

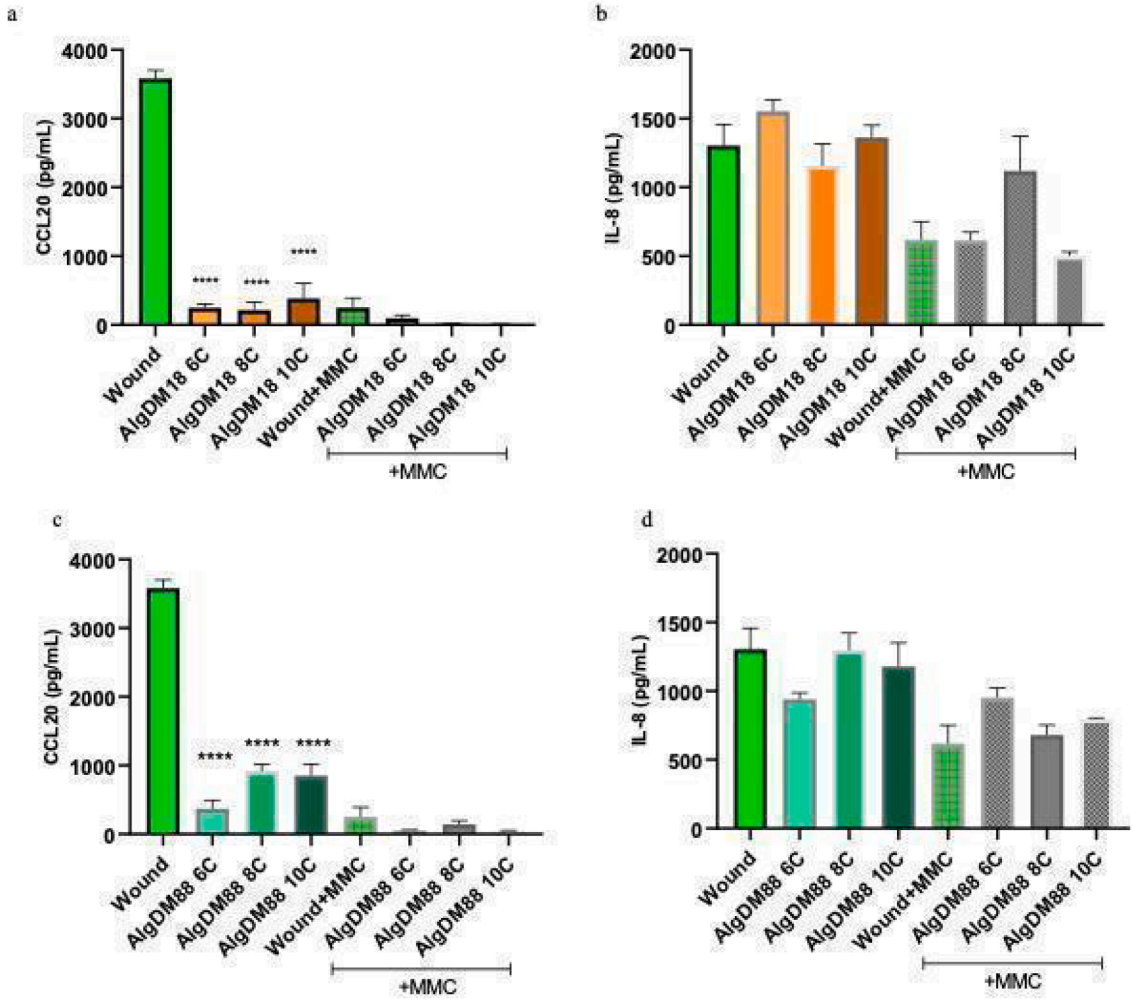

Fig. 2. CCL20 and IL-8 protein production by pre-treatment with empty alginate-pectin-based beads on non-MMC and MMC T84 cell monolayer. IL-6, IL-13, IL28a, and IL-33 were also quantified but not detectable in the supernatant. The non-MMC and MMC T84 cell monolayer was pre-treated with empty Alginate-pectin-based beads for 24 hours, and then cell was scratched, followed by CCL20 and IL-8 quantification in cell supernatants after 48 hours. The secretion of CCL20 (**a**) and IL-8 (**b**) by scratched T84 cell monolayer pre-treated with AlgDM18 empty beads (n = 5), and the secretion of CCL20 (**c**) and IL-8 (**d**) by scratched T84 cell monolayer pre-treated with AlgDM88 empty beads (n = 5). Data are expressed as mean ± SEM. Statistical differences were shown compared with wound control. Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001).

L. plantarum WCFS1 in AlgDM88 beads did show a tendency to increase CCL20 production in the MMC T84 cell monolayer. The pre-treatment with *L. plantarum WCFS1* in alginate-pectin-based beads lowered the release of IL-8 on the non-MMC T84 cell monolayer, meanwhile increasing the secretion of IL-8 on MMC T84 cell monolayer.

The pre-treatment with either amount of *L. plantarum WCFS1* in AlgDM18 beads did lower the secretion of CCL20 48 hours after the scratch ($p<0.0001$) in non-MMC T84 cell monolayers. It was 10 times lower. There were no differences in the production of CCL20 in MMC T84 cell monolayer (Fig. 4**a**). *L. plantarum* WCFS1 encapsulated in AlgDM88 beads significantly decreased CCL20 production in non-MMC T84 cell monolayers 48 hours after scratching ($p<0.0001$), though the reduction was less pronounced with AlgDM88 6c beads, resulting in approximately a fourfold decrease (Fig. 4**c**). For IL-8, *L. plantarum* WCFS1 encapsulated in AlgDM18 6c and 8c beads lowered production in non-MMC T84 cell monolayers to 618.7 ± 131.7 pg/mL ($p<0.05$) and 874.7 ± 57.6 pg/mL ($p<0.0001$), respectively, after 48 hours. In MMC T84 cell monolayers, AlgDM18 6c beads decreased IL-8 production to 185.3 ± 45.2 pg/mL ($p<0.05$), while AlgDM18 8c beads led to an increase in IL-8 secretion to 1128 ± 55.9 pg/mL ($p<0.01$) 48 hours post-scratching (Fig. 4**b**).The production of IL-8 was reduced by *L. plantarum WCFS1* in AlgDM88 10c pre-treatment to 440 pg/mL ($p<0.05$) in non-MMC T84 cell monolayers, meanwhile, the pre-treatment with both *L. plantarum WCFS1* in AlgDM88 6c and 8c did enhance the production of IL-8 to 1473 ± 102.2 pg/mL ($p<0.05$) and 2902 ± 315.8 pg/mL ($p<0.0001$) in MMC T84 cell monolayer after 48 hours scratching (Fig. 4**d**).

3.5. Empty chitosan-DM18 pectin beads impair cell proliferation and migration meanwhile empty chitosan-DM88 pectin beads improve cell proliferation

Next the experiments were repeated with a different beads system proposed for delivering living bacteria to lower parts of the gastrointestinal tract, *i.e.* chitosan-pectin beads. The confluent T84 epithelial cell monolayers were pre-incubated for 24 hours with 6, 8, or 10 beads of chitosan-pectin-based beads in non-MMC and MMC T84 cell monolayers after 48 hours of scratching as described previously.

The ChitoDM18 empty beads pre-treatment reduced cell proliferation in non-MMC T84 cell monolayer and cell migration in MMC T84 cell monolayer after 48 of scratching. On the other hand, ChitoDM88 empty beads pre-treatment increased cell proliferation in non-MMC T84 cell monolayer and did not affect cell migration of the MMC T84 gut epithelial cell monolayer.

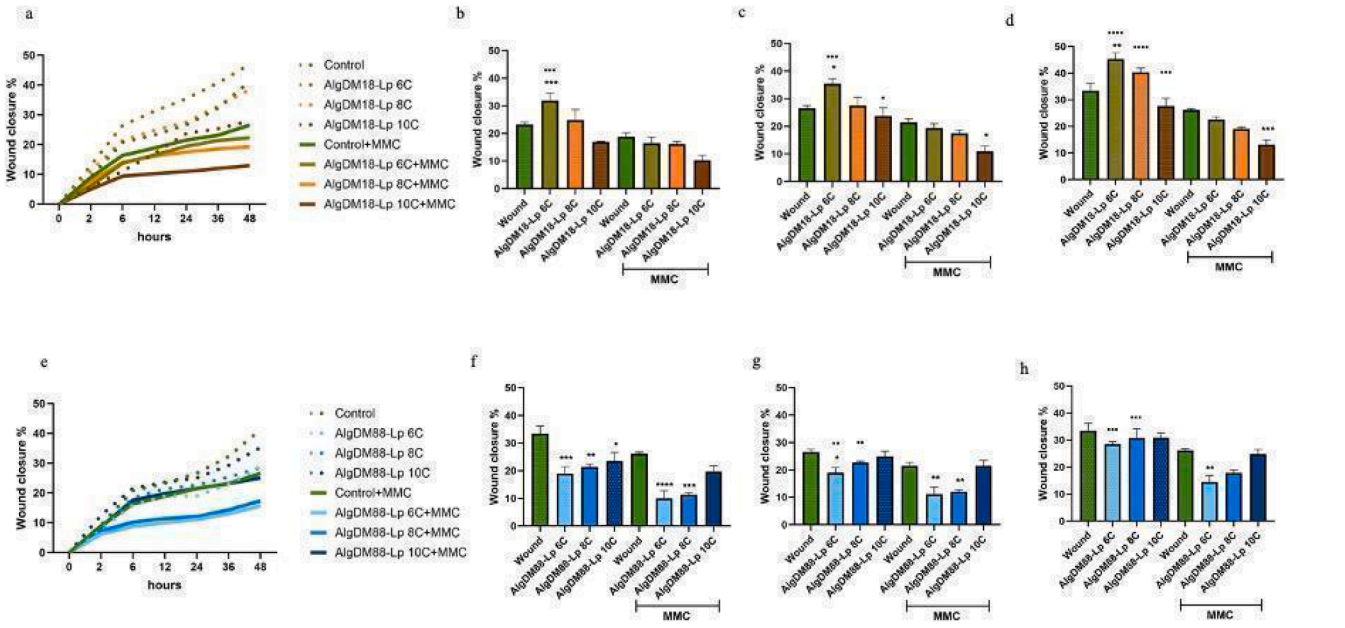

Fig. 3. The impact of *L. plantarum WCFS1* in alginate-pectin-based beads on cell proliferation and cell migration. **a**) Wound closure was measured over 48 hours on non-MMC and MMC T84 cell monolayer after the pre-treatment of *L. plantarum WCFS1* in AlgDM18 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of AlgDM18 at 12 (**b**), 24 (**c**) and 48 hours (**d**) after the scratch assay. **e**) Wound closure was measured over 48 hours on untreated and pre-treated mitomycin T84 cell monolayer after the pre-treatment of *L. plantarum WCFS1* in AlgDM88 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of AlgDM88 at 12 (**f**), 24 (**g**) and 48 hours (**h**) after the scratch assay. Data are expressed as mean ± SEM. Groups were compared with wound control and presented as %. N=5 independent experiments with two technical replicates for each condition Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

The proliferation of non-MMC T84 cell monolayers was delayed by pre-treatment with ChitoDM18 8c empty beads. At 24 and 48 hours, proliferation was reduced to 18.2 ± 0.2% ($p<0.01$) and 23.4 ± 2.1% ($p\leq0.001$), respectively (Fig. 5**a–d**). In contrast, cell migration was significantly impaired by all concentrations of empty ChitoDM18 beads. At 12 hours, migration decreased to 9.0 ± 2.1% ($p\leq0.01$) and 7.3 ± 2.2% ($p\leq0.001$) with 8c and 10c beads, respectively. At 24 hours, migration was reduced to 14.3 ± 1.1% ($p\leq0.01$) with 6c beads, 10.4 ± 2.1% ($p\leq 0.0001$) with 8c beads, and 11.5 ± 1.6% ($p\leq0.001$) with 10c beads. By 48 hours, migration further decreased to 17.5 ± 1.3% ($p\leq0.01$) with 6c beads, 13.6 ± 1.0% ($p\leq0.0001$) with 8c beads, and 18.3 ± 0.9% ($p\leq0.01$) with 10c beads in MMC T84 cell monolayers (Fig. 5**a–d, Fig. S6**). These results indicate that pre-treatment with ChitoDM18 empty beads slowed cell migration compared to the control. However, pre-treatment with ChitoDM88 empty beads promoted cell proliferation compared to the control.

The ChitoDM88 6c empty beads enhanced cell proliferation in non-MMC T84 cell monolayers. Proliferation increased to 35.5 ± 1.6% ($p\leq0.05$) at 12 hours, 36.2 ± 0.7% at 24 hours, and 44.8 ± 0.6% ($p\leq0.01$) at 48 hours post-scratch (Fig. 5**e–h, Fig. S7**). However, pre-treatment with any concentration of ChitoDM88 empty beads did not improve migration in MMC T84 cell monolayers. While ChitoDM88 10c beads showed a tendency to reduce migration at 12 hours, this effect was not statistically significant (Fig. 5**e–h**). When comparing non-MMC and MMC T84 cell monolayers treated with the same concentrations of ChitoDM88 6c and 8c beads, notable differences were observed (Fig. 5**e–h, Fig. S7**). These findings suggest that ChitoDM88 primarily enhances cell proliferation rather than migration.

3.6. Empty chitosan-DM88 pectin beads decrease secretion of CCL20 and IL-8

Next, we determined whether chitosan-pectin-based beads affected the epithelial immune responses by quantifying CCL20, IL-6, IL-8, IL-13, IL28a, and IL-33 in the supernatant of non-MMC and MMC T84 cell monolayers after 48 hours of scratching as described above.

Pre-treatment with ChitoDM18 and ChitoDM88 empty beads reduced CCL20 production by non-MMC T84 cell monolayers. However, IL-8 secretion was unaffected by ChitoDM18 empty beads but was decreased by ChitoDM88 empty beads in non-MMC T84 cell monolayers. Specifically, pre-treatment with any concentration of ChitoDM18 empty beads significantly decreased CCL20 secretion ($p\leq0.0001$) by non-MMC T84 cell monolayers 48 hours after scratching, with levels approximately ten times lower than the control. In contrast, ChitoDM18 8c beads reduced CCL20 secretion by around fivefold compared to the control (Fig. 6**a**). Chitosan-DM18 empty beads pre-treatment did not increase the release of CCL20 by MMC T84 cell monolayers (Fig. 6**c**). The pre-treatment with either amount of ChitoDM88 empty beads reduced the release of CCL20 ($p\leq0.0001$) by non-MMC T84 cell monolayers after 48 hours of scratching, which was around 7 times lower than the control. No differences were observed in the MMC T84 cell monolayer after 48 hours of scratching (Fig. 6**b**).

The secretion of IL-8 was not significantly affected by any concentration of ChitoDM18 empty beads in either non-MMC or MMC T84 cell monolayers 48 hours after scratching. However, a tendency for increased IL-8 release, approximately twofold, was observed with ChitoDM18 6c and 8c beads in MMC T84 cell monolayers. In contrast, pre-treatment with ChitoDM88 6c empty beads reduced IL-8 secretion in non-MMC T84 cell monolayers to 665.7 ± 102.7 pg/mL, which is approximately twofold lower than the control ($p\leq0.01$) 48 hours post-scratching. Meanwhile, IL-8 secretion was not influenced by ChitoDM88 empty beads in MMC T84 cell monolayers (Fig. 6**d**).

3.7. L. plantarum WCFS1 on chitosan-DM18 beads stimulates cell proliferation but inhibits migration, while chitosan-DM88 beads mainly reduce migration

We next investigated the effects of pre-treatment with empty chitosan-pectin-based beads on cell proliferation and migration using a wound healing assay. This allowed us to assess whether the chitosan-

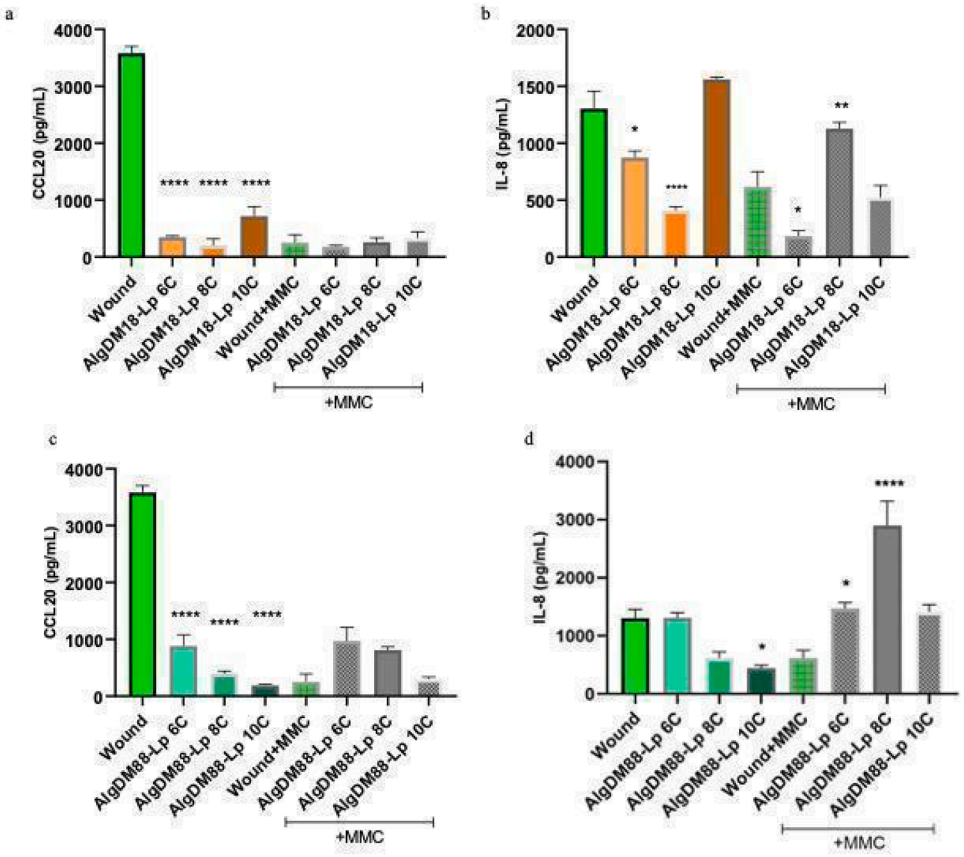

Fig. 4. CCL20 and IL-8 protein quantification by pre-treatment with *L. plantarum WCFS1* in alginate-pectin-based beads on non-MMC and MMC T84 cell monolayer. IL-6, IL-13, IL28a, and IL-33 were also quantified but not detectable in the supernatant. The non-MMC and MMC T84 cell monolayer was pre-treated with empty Alginate-pectin-based beads for 24 hours, and then cell was scratched, followed by CCL20 and IL-8 quantification in cell supernatants after 48 hours. The secretion of CCL20 (**a**) and IL-8 (**b**) by scratched T84 cell monolayer pre-treated with *L. plantarum WCFS1* in AlgDM18 beads (n = 5), and the secretion of CCL20 (**c**) and IL-8 (**d**) by scratched T84 cell monolayer pre-treated with *L. plantarum WCFS1* in AlgDM88 beads (n = 5). Data are expressed as mean ± SEM. Statistical differences were shown compared with wound control. Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001).

pectin beads could influence how *L. plantarum WCFS1* impacts wound healing. Specifically, we examined how incorporating *L. plantarum WCFS1* into chitosan-pectin beads affects cell behavior. To test this, *L. plantarum WCFS1* was loaded into ChitoDM18 and ChitoDM88 beads. Sets of 6, 8, and 10 beads were then applied to both non-MMC and MMC-treated T84 cell monolayers, following the experimental setup described earlier.

The effects of *L. plantarum WCFS1* in chitosan-based beads on cell behavior were distinct between bead types. *L. plantarum WCFS1* in ChitoDM18 beads significantly enhanced cell proliferation while reducing cell migration. In contrast, *L. plantarum WCFS1* in ChitoDM88 beads had no effect on cell proliferation but decreased cell migration. Pre-treatment with *L. plantarum WCFS1* in ChitoDM18 10c beads further demonstrated a strong proliferative effect in non-MMC-treated T84 cell monolayers. Cell proliferation increased to 33.3 ± 1.6% ($p \leq 0.001$) at 12 hours, 37.6 ± 1.2% ($p \leq 0.01$) at 24 hours, and 45.0 ± 1.4% ($p \leq 0.01$) at 48 hours (Fig. 7**a, b, c, d, Fig. S8**). However, pre-treatment with *L. plantarum WCFS1* in ChitoDM18 6c reduced the cell migration in MMC T84 cell monolayer to 9.4 ± 1.3% ($p \leq 0.001$) at 12 hours, 10.9 ± 0.2% ($p \leq 0.001$) at 24 hours and 13.6 ± 0.8% ($p \leq 0.001$) at 48 hours (Fig. 7**a, b, c, d, Fig. S8**). When comparing non-MMC and MMC-treated T84 cell monolayers exposed to the same number of beads, *L. plantarum WCFS1* in ChitoDM18 10c beads showed significant differences in effects on gut epithelial cells at 12, 24, and 48 hours ($p \leq 0.0001$; Fig. 7**a–d, Fig. S8**). These results suggest that *L. plantarum WCFS1* in ChitoDM18 10c beads primarily enhances cell proliferation rather than migration. In contrast, pre-treatment with *L. plantarum WCFS1* in ChitoDM88 beads, regardless of the number of beads used, did not improve cell proliferation in non-MMC-treated T84 gut epithelial cell monolayers after 48 hours of the scratch assay (Fig. 7**e–h**). However, pre-treatment with ChitoDM88 6c and 8c beads significantly reduced cell migration in MMC-treated T84 cell monolayers. Migration decreased to 8.3 ± 1.2% ($p \leq 0.0001$) and 7.2 ± 0.9% ($p \leq 0.0001$) at 12 hours, 10.5 ± 1.8% ($p \leq 0.0001$) and 11.0 ± 1.3% ($p \leq 0.001$) at 24 hours, and 11.9 ± 1.0% ($p \leq 0.0001$) and 13.5 ± 1.7% ($p \leq 0.001$) at 48 hours (Fig. 7**e–h, Fig. S9**). Differences were also observed between non-MMC and MMC-treated T84 cell monolayers for *L. plantarum WCFS1* in ChitoDM88 6c and 8c beads at 12 to 48 hours and with 10c beads at 48 hours post-scratch (Fig. 7**e–h**). These findings indicate that *L. plantarum WCFS1* in ChitoDM88 beads may promote cell proliferation rather than migration.

3.8. L. plantarum WCFS1-loaded chitosan-pectin-based beads reduce the secretion of CCL20 on cell proliferation and L. plantarum WCFS1 in chitosan/pectin DM18 reduces IL-8 secretion

Afterwards, we determined whether *L. plantarum WCFS1* in chitosan-pectin-based beads affected the epithelial immune responses by quantifying CCL20, IL-6, IL-8, IL-13, IL28a, and IL-33 in the supernatant of non-MMC and MMC T84 cell monolayer after 48 hours of scratching as described above.

L. plantarum WCFS1 in chitosan-pectin-based beads did reduce the secretion of CCL20 in non-MMCT T84 cell monolayer. However,

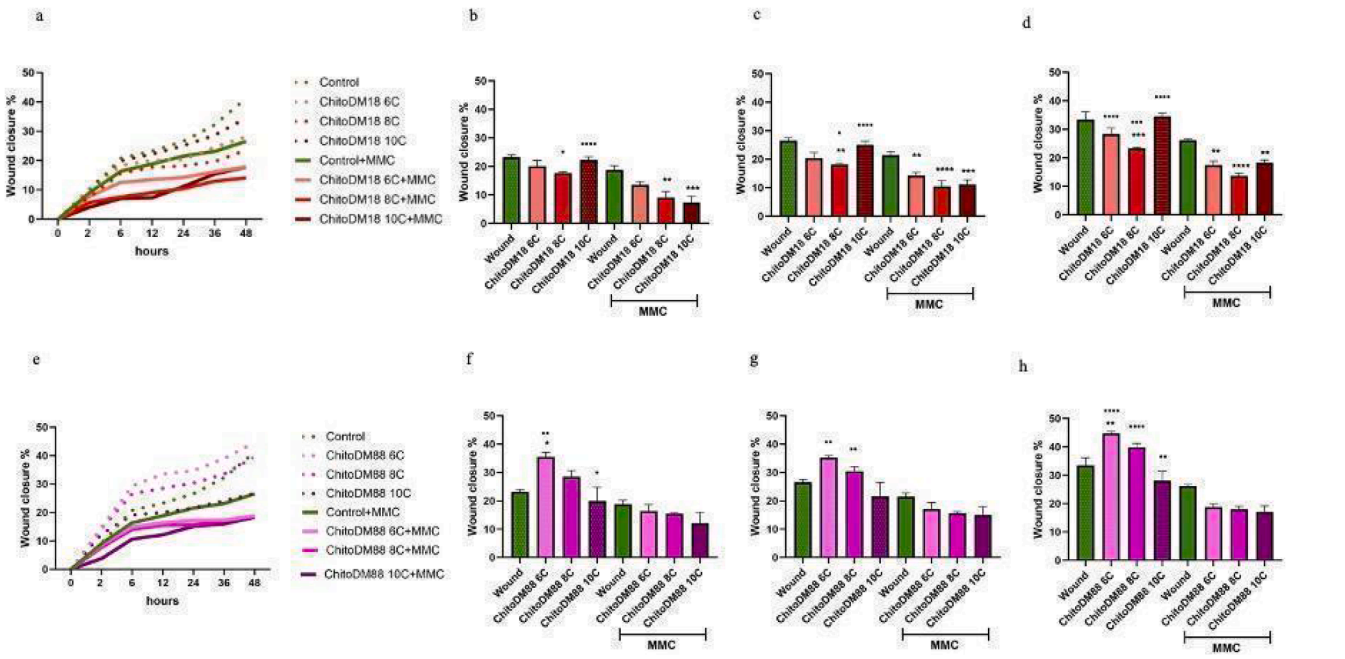

Fig. 5. The effect of empty chitosan-pectin-based beads on cell proliferation and cell migration. **a**) Wound closure was measured over 48 hours on non-MMC and MMC T84 cell monolayer after the pre-treatment of ChitoDM18 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of ChitoDM18 at 12 (**b**), 24 (**c**) and 48 hours (**d**) after the scratch assay. **e**) Wound closure was measured over 48 hours on untreated and pre-treated mitomycin T84 cell monolayer after the pre-treatment of ChitoDM88 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of AlgDM88 at 12 (**f**), 24 (**g**) and 48 hours (**h**) after the scratch assay. Data are expressed as mean ± SEM. Groups were compared with wound control and presented as %. N=5 independent experiments with two technical replicates for each condition Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

L. plantarum WCFS1 in ChitoDM88 beads did tend to increase CCL20 production in the MMC T84 cell monolayer. The pre-treatment with *L. plantarum WCFS1* in ChitoDM18 beads lowered the release of IL-8 in the non-MMC and MMC T84 cell monolayers, while *L. plantarum WCFS1* in ChitoDM88 did not impact IL-8 release. The pre-treatment with either amount of *L. plantarum WCFS1* in ChitoDM18 beads lowered the secretion of CCL20 ($p \leq 0.0001$) in non-MMC T84 cells after 48 hours of the scratch. The reduction was approximately 25-fold. (Fig. 8**a**). *L. plantarum WCFS1* in ChitoDM18 8c in MMC T84 cell monolayers tended to increase CCL20 production. This was 4 times higher than the control. Pre-treatment with *L. plantarum WCFS1* in ChitoDM88 beads (6c, 8c, and 10c) significantly reduced CCL20 secretion in non-MMC T84 cells 48 hours after the scratch. Levels dropped to 582.5 ± 128.6 pg/mL ($p \leq 0.01$), 1012 ± 276.1 pg/mL ($p \leq 0.05$), and 1009 ± 248.8 pg/mL ($p \leq 0.05$), approximately three times lower than the control. However, in MMC-treated T84 cell monolayers, pre-treatment with ChitoDM88 6c and 10c beads tended to increase CCL20 release by about six times compared to the control (Fig. 8**c**). For IL-8, pre-treatment with *L. plantarum WCFS1* in ChitoDM18 beads (any amount) reduced its secretion in both non-MMC and MMC T84 cell monolayers by approximately 15-fold after 48 hours ($p \leq 0.0001$; Fig. 8**b**). In contrast, no significant differences in IL-8 production were observed in T84 monolayers pre-treated with ChitoDM88 beads. However, there was a tendency for IL-8 secretion to increase by about three times in MMC-treated T84 cell monolayers compared to the control (Fig. 8**d**).

4. Discussion

Wound healing and the regulation of the epithelial barrier function are essential processes to maintain intestinal epithelial homeostasis. Wound repair is essential for re-establishing the mucosal epithelial barrier and restoring intestinal homeostasis, which are critical for the efficient resolution of inflammation (Iizuka & Konno, 2011; Sommer et al., 2021). This study aimed to evaluate the effects of pectin's degree of methylation on wound healing, specifically on cell proliferation and migration, using pectin-based microcapsules. Additionally, we investigated the effects of encapsulated *Lactobacillus plantarum* WCFS1 within these pectin-based microbeads. Alginate and chitosan were used as stabilizing agents for the microcapsules. The study focused on understanding how both the pectin DM and the encapsulated *L. plantarum* influence wound healing processes.

This study shows that proliferation and migration in gut epithelial wound healing are differently regulated and that the extent to which it is regulated is both bead-type and pectin DM dependent. Proliferation and migration are to different extents regulated by adding *L. plantarum* in the pectin-based beads. DM88 pectin-based empty beads improved cell proliferation rather than cell migration. Beads containing DM88 pectin even lowered cell migration. Adding *L. plantarum* to the beads resulted in differences in cell proliferation and migration in the wound healing process. Pre-treatment with *L. plantarum* in DM18 lemon pectin-based beads enhanced cell proliferation without delaying cell migration. However, an exception was observed with *L. plantarum* in ChitoDM18 6C beads, where a decrease in cell migration was noted. These findings are summarized in Fig. 9.

Our results demonstrate that CCL20 is decreased by DM88 pectin-containing capsules and by DM18 pectin-containing capsules loaded with *Lactobacillus plantarum*. CCL20 plays a pivotal role in wound healing by stimulating cell proliferation, migration, and immune cell recruitment (McCully et al., 2018). It is highly inducible in response to inflammatory stimuli, a finding that aligns with our observations. Alongside IL-8, CCL20 accelerates epithelial repair by facilitating essential processes such as actin cytoskeleton reorganization and myosin light chain phosphorylation, which are critical for wound closure (Iizuka & Konno, 2011; Furue et al., 2020). CCL20 also supports intestinal homeostasis by recruiting CCR6-expressing immune cells to the gut mucosa, thereby maintaining barrier integrity and promoting tissue repair (Kwon, 2002). Notably, its role is dualistic and dependent on the tissue-circumstances. Under normal conditions, CCL20 functions as a

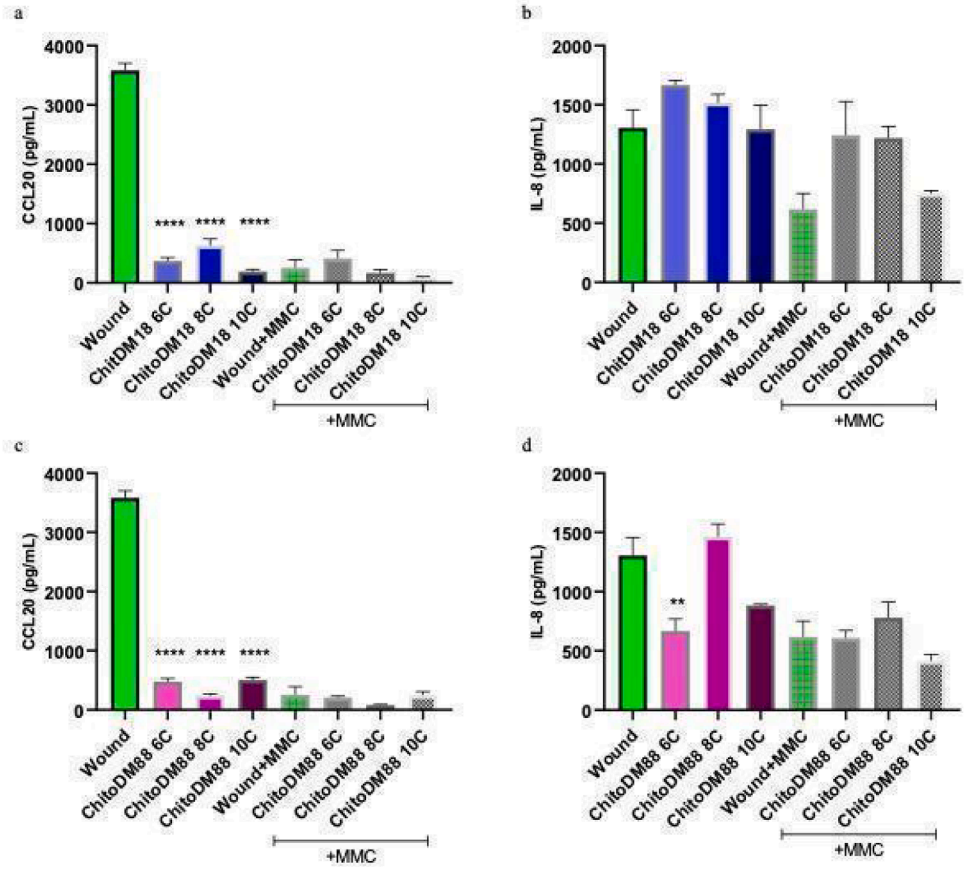

Fig. 6. CCL20 and IL-8 protein production by pre-treatment with empty chitosan-pectin-based beads on non-MMC and MMC T84 cell monolayer. IL-6, IL-13, IL28a, and IL-33 were also quantified but not detectable in the supernatant. The non-MMC and MMC T84 cell monolayer was pre-treated with empty Chitosan-pectin-based beads for 24 hours, and then cell was scratched, followed by CCL20 and IL-8 quantification in cell supernatants after 48 hours. The secretion of CCL20 (**a**) and IL-8 (**b**) by scratched T84 cell monolayer pre-treated with ChitoM18 empty beads (n = 5), and the secretion of CCL20 (**c**) and IL-8 (**d**) by scratched T84 cell monolayer pre-treated with ChitoDM88 empty beads (n = 5). Data are expressed as mean ± SEM. Statistical differences were shown compared with wound control. Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

homeostatic molecule, yet its pro-inflammatory nature can drive excessive proliferation and migration, as observed in colorectal cancer models (Nandi et al., 2021; Yue et al., 2024). Interestingly, our findings suggest that pectin-based beads modulate CCL20 release. While this supports epithelial repair and immune cell recruitment, it may also offer a strategy to control excessive cell proliferation and migration in disease contexts such as colorectal cancer. These results underscore the multifaceted role of CCL20 in coordinating inflammation, tissue repair, and intestinal homeostasis.

Our findings show that most pectin-based beads reduced or had no effect on IL-8 release by intestinal epithelial cells. Interestingly, DM18 pectin-containing beads loaded with *L. plantarum* further decreased IL-8 secretion. This reduction may be linked to delayed cell migration and to reduced neutrophil recruitment *in vivo*, both of which are essential for efficient wound closure. IL-8, a multifunctional member of the α-chemokine family, is a potent stimulator of neutrophil activation and chemotaxis. Within the intestinal mucosa, IL-8 guides neutrophils to sites of injury and inflammation, where they are the first immune cells to infiltrate and support tissue repair (Brazil et al., 2019; Sturm et al., 2005). Neutrophils play a dual role during wound healing. While their timely recruitment is crucial for clearing pathogens and releasing growth factors, excessive IL-8-driven neutrophil accumulation can exacerbate inflammation and hinder epithelial repair. The modulation of IL-8 secretion by pectin-based beads in our study highlights a potential mechanism for regulating inflammation while supporting cell proliferation. Importantly, the observed reduction in IL-8 aligns with our earlier findings on CCL20, suggesting that pectin-based beads may shift the balance toward proliferation rather than migration by promoting CCL20 signaling while suppressing IL-8 release. These findings indicate that pectin-based beads, particularly those loaded with *L. plantarum*, can fine-tune the inflammatory response. By reducing IL-8 secretion, they may limit excessive neutrophil infiltration, thus promoting a more controlled and efficient wound healing process. This nuanced modulation of cytokine activity underscores the potential of pectin-based systems to balance inflammation, proliferation, and migration during epithelial repair.

Our study shows that DM88 pectin-based empty beads and DM18 pectin-based beads loaded with *L. plantarum* significantly improved cell proliferation. This suggests their potential to prevent the loss of gut homeostasis in inflammatory bowel diseases or to support for example post-surgical gut healing. Maintaining intestinal homeostasis requires a precise balance between proliferating and non-proliferating cells, as even minor imbalances can result in uncontrolled growth or barrier dysfunction (Huelsz-Prince et al., 2022; Gall et al., 2023). Previous studies have shown that ChitoDM88 empty beads and *L. plantarum* loaded pectin-based beads prevent barrier disruption (Galvez-Jiron et al., 2025), reinforcing our findings that pectin-based systems support epithelial integrity. Dysregulation of cell proliferation, migration, and shedding contributes to conditions such as inflammatory bowel diseases, microbial infections, and metabolic disorders (Chelakkot et al., 2018), which could be ameliorated and prevented by pectin-based beads. Our findings suggest that pectin-based beads support this balance, enhancing epithelial proliferation while regulating key cytokines to promote efficient wound repair and maintain gut homeostasis.

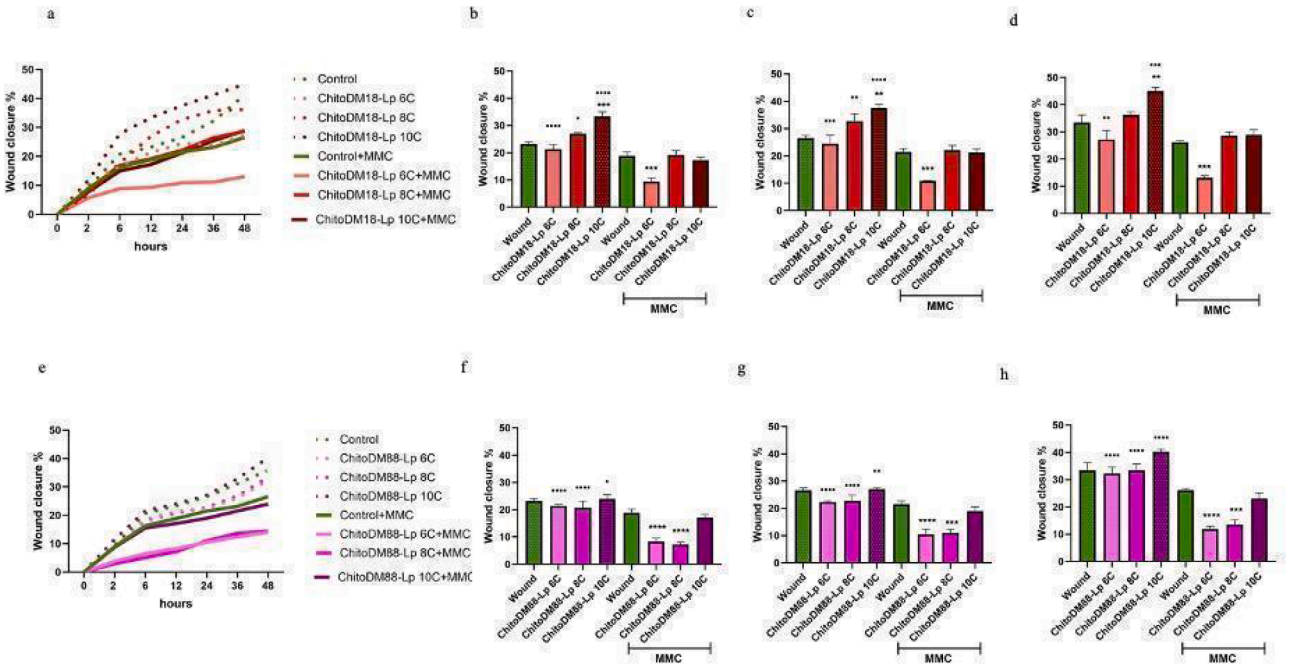

Fig. 7. The impact of *L. plantarum WCFS1* in chitosan-pectin-based beads on cell proliferation and cell migration. **a**) Wound closure was measured over 48 hours on non-MMC and MMC T84 cell monolayer after the pre-treatment of *L. plantarum WCFS1* in ChitoM18 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of ChitoDM18 at 12 (**b**), 24 (**c**) and 48 hours (**d**) after the scratch assay. **e**) Wound closure was measured over 48 hours on untreated and pre-treated mitomycin T84 cell monolayer after the pre-treatment of *L. plantarum WCFS1* in ChitoDM88 6, 8, and 10 beads. Wound closure on non-MMC and MMC T84 cell monolayers after the pre-treatment of ChitoDM88 at 12 (**f**), 24 (**g**) and 48 hours (**h**) after the scratch assay. Data are expressed as mean ± SEM. Groups were compared with wound control and presented as %. N=5 independent experiments with two technical replicates for each condition Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

Our study shows that epithelial cell proliferation and migration are influenced by both bead composition and pectin degree of methylation (DM), as well as the concentration of the beads. Empty DM88 pectin-based beads promoted cell proliferation at higher concentrations, while AlgDM88 beads enhanced proliferation at lower concentrations compared to ChitoDM88. These effects may be due to the interaction between DM88 pectin, different charge and composition of stabilizing agents, and epithelial receptors, along with the stronger mucoadhesion of high DM pectin to the epithelial cells, which supports wound closure (Thirawong et al., 2007). Interestingly, higher concentrations of AlgDM88 delayed cell migration beyond 24 hours, suggesting a potential strategy to limit cell migration in colorectal cancer. Incorporating *L. plantarum WCFS1* into DM18 pectin-based beads altered these trends. Loaded AlgDM18 beads enhanced cell proliferation at lower concentrations, whereas *L. plantarum* loaded ChitoDM18 promoted proliferation at higher concentrations, potentially due to the antibacterial activity of chitosan affecting *L. plantarum* metabolism. However, higher concentrations of loaded AlgDM18 delayed cell migration beyond 24 hours, while lower concentrations of *L. plantarum* loaded ChitoDM18 caused earlier migration delays at 12 hours. Our results highlight the unique effects of bead type, bacterial loading, and concentration on balancing proliferation and migration during epithelial repair.

Our study reveals a differential effect when alginate and chitosan are used as stabilizing agents in pectin-based beads. To provide inflammatory conditions a scratch wound assay on T84 human colonic epithelial monolayers was used, due this model mimics epithelial injury and has been widely accepted as a proxy for assessing epithelial repair mechanisms and immune responses, particularly cytokine release, in the absence of added proinflammatory agents (Grada et al., 2017; Brazil et al., 2019). Scratching induces mechanical disruption of the monolayer, which leads to the activation of intrinsic wound healing pathways and cytokine production, including IL-8 and CCL20, as part of the innate epithelial response (Fujiie et al., 2001; Furue et al., 2020). These cytokines are known to be upregulated in response to physical injury and contribute to epithelial restitution and immune cell recruitment. Thus, although no exogenous inflammatory mediators (e.g., TNF-α, LPS) were added, the model elicits a reproducible pro-inflammatory state associated with mechanical epithelial damage, which is biologically relevant for assessing the immunomodulatory effects of pectin-based beads and *Lactobacillus plantarum.* DM88 pectin-based beads supported cell proliferation, while empty alginate-pectin beads did not influence IL-8 secretion under non-mitomycin-C conditions. In contrast, *L. plantarum* loaded alginate-pectin beads increased IL-8 production under non-mitomycin-C conditions but decreased IL-8 release in mitomycin-C conditions. Empty AlgDM18 beads had no effect on cell proliferation or migration. These results suggest that alginate-pectin beads may delay cell migration by reducing IL-8 secretion, thereby modulating the inflammatory response, as previously observed for alginate-based matrices that attenuate IL-1β, IL-6, and IL-8 levels (Qiu et al., 2024). Empty chitosan-based beads did not affect IL-8 production under mitomycin-C conditions. However, *L.plantarum* loaded ChitoDM18 beads reduced IL-8 secretion under both non-mitomycin-C and mitomycin-C conditions. This effect aligns with the known anti-inflammatory properties of chitosan oligosaccharides, which downregulate IL-1β, IL-6, and IL-8 in inflammatory bowel disease (Jhundoo et al., 2020). IL-8 plays a central role in intestinal inflammation and colorectal cancer progression. By decreasing IL-8 secretion, both alginate- and chitosan-based pectin beads may regulate the inflammatory response, alleviate neutrophil-driven mucosal injury, and delay cell migration in colorectal cancer. These findings highlight the therapeutic potential of pectin-based systems for maintaining immune homeostasis and mitigating intestinal inflammation. Angiogenesis, granulation tissue development, and re-epithelialization are just a few of the processes supported by cell migration in the wound healing process. Fibroblasts, microvascular endothelial cells, and immune cells work together to regulate damage healing with several growth factors

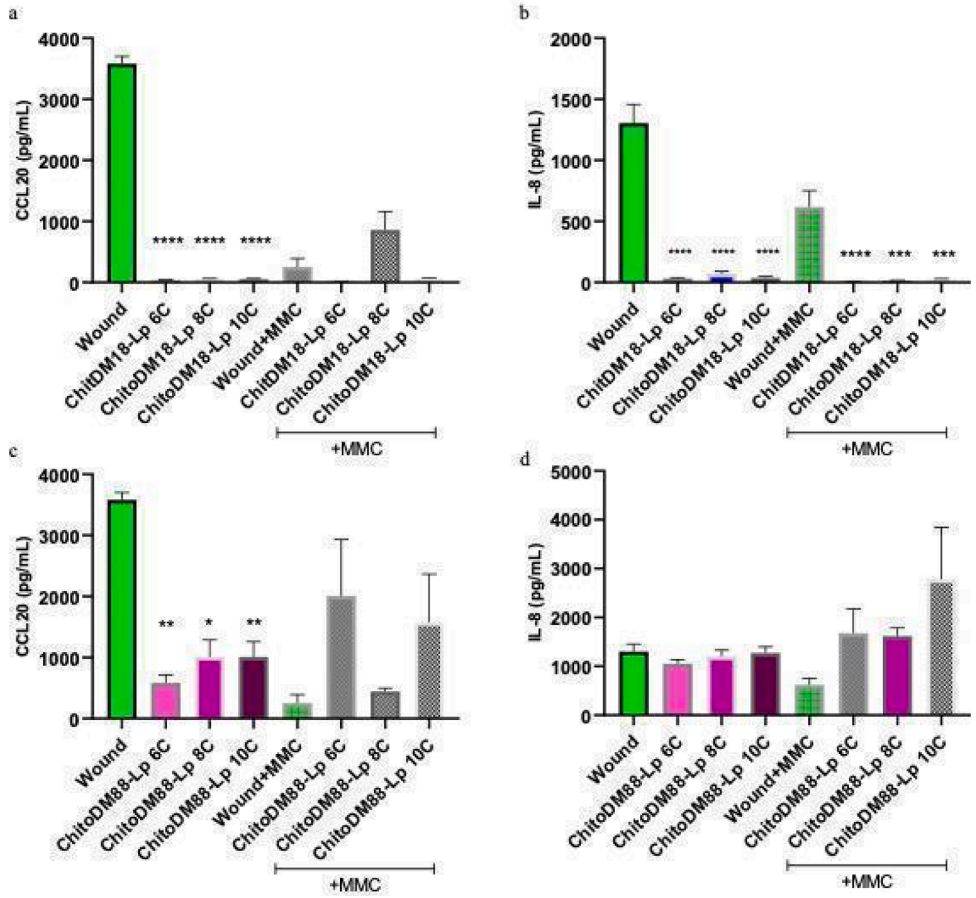

Fig. 8. CCL20 and IL-8 protein quantification by pre-treatment with *L. plantarum WCFS1* in chitosan-pectin-based beads on non-MMC and MMC T84 cell monolayer. IL-6, IL-13, IL28a, and IL-33 were also quantified but not detectable in the supernatant. The non-MMC and MMC T84 cell monolayer was pre-treated with empty Chitosan-pectin-based beads for 24 hours, and then cell was scratched, followed by CCL20 and IL-8 quantification in cell supernatants after 48 hours. The secretion of CCL20 (**a**) and IL-8 (**b**) by scratched T84 cell monolayer pre-treated with *L. plantarum WCFS1* in ChitoDM18 beads (n = 5), and the secretion of CCL20 (**c**) and IL-8 (**d**) by scratched T84 cell monolayer pre-treated with *L. plantarum WCFS1* in ChitoDM88 beads (n = 5). Data are expressed as mean ± SEM. Statistical differences were shown compared with wound control. Statistical differences were processed with a one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$).

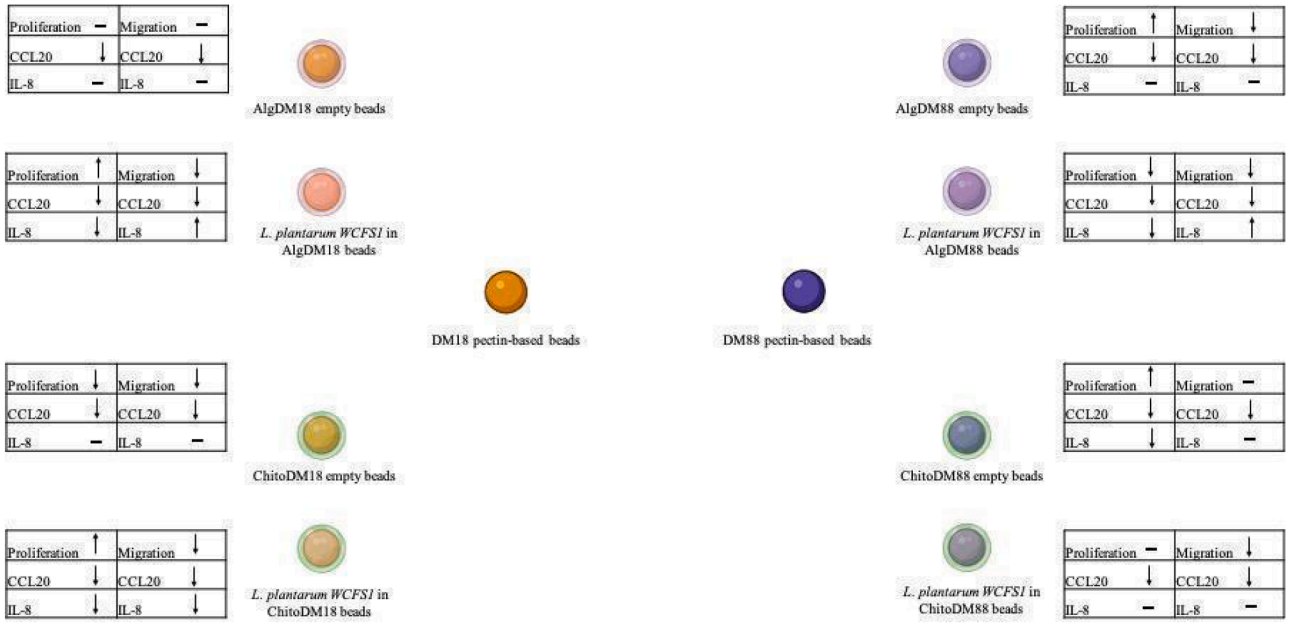

Fig. 9. Summary figure describing the main findings of the current paper.

(Martinotti, S., & Ranzato, E. (2020), processes which are not applied in scratch monolayer assay, therefore tridimensional cell culture models or *in vivo* models might be used in the future to test the effects on proliferation and migration by the microbeads.

Our results on enhanced wound healing and anti-inflammatory capacity of pectin-based delivery systems corroborate the findings of others. In this study microencapsulated *Lactobacillus rhamnosus* in sodium alginate microbeads was administered to healthy mice over a six-week period. Serum analysis revealed significant reductions in total oxidant status, total antioxidant status, and C-reactive protein levels, underscoring a systemic anti-inflammatory response (Madali-Kafes et al., 2025). These outcomes are consistent with earlier studies showing that encapsulated probiotics achieve markedly higher intestinal colonization which may mount up to 17-fold compared to non-encapsulated forms (Wang et al., 2020). Advanced encapsulation techniques, such as nanocoating, have been shown to improve both adhesion and protective function, leading to synergistic effects on inflammation reduction and intestinal barrier restoration (Xu et al., 2022; Pan et al., 2022). Complementing our own results, *Lactobacillus paracasei* microencapsulated on starch pellets enhanced mucin secretion, increased sIgA levels, and upregulated mRNA expression of MUC-2 and tight junction proteins in murine models, thereby reinforcing epithelial integrity and reducing pro-inflammatory markers (Gyawali et al., 2023). Taken together, these data support our conclusion that the ChitoDM88-Lp system not only facilitates targeted probiotic delivery but may also promote long-term colonization and mucosal healing, driven by the strong mucoadhesive properties of high-DM pectins used in our formulations.

In summary, this study highlights the distinct effects of *L. plantarum* WCFS-1 encapsulated in pectin-based beads on gut epithelial wound healing. Specifically, empty DM88 pectin-based beads supported cell proliferation at higher concentrations but delayed migration. Incorporating *L. plantarum* into the DM18 beads enhanced cell proliferation at lower concentrations while modulating immune responses, such as reducing CCL20 production and altering IL-8 secretion. These findings suggest that pectin-based encapsulation systems can influence cellular behavior in a dose-dependent manner, tailored by the degree of methylation and stabilizing agent. The therapeutic potential of *L. plantarum* encapsulated in pectin-based beads is promising for disorders involving epithelial barrier dysfunction and chronic inflammation. Examples include inflammatory bowel diseases, where epithelial damage and barrier disruption are prominent, and post-operative healing of intestinal wounds, as previously discussed in related studies (Kwon, 2002; Galvez-Jiron et al., 2025). Additionally, the immunomodulatory properties of these beads, particularly their ability to modulate IL-8 and CCL20 secretion, position them as potential adjuncts for managing colorectal cancer or radiation-induced colitis, where cytokine regulation is crucial (Nandi et al., 2021; Liu et al., 2001). Further research should explore *in vivo* applications to validate these therapeutic possibilities and optimize bead formulations for targeted clinical outcomes. The clinical application of pectin-based encapsulation systems containing *Lactobacillus plantarum* requires addressing several translational barriers. Key challenges include ensuring biocompatibility and mechanical stability during gastrointestinal transit, as well as maintaining structural integrity under physiological conditions (Jia et al., 2023). Moreover, large-scale production must overcome issues related to reproducibility and quality control, which remain critical for regulatory approval. Personalized formulations tailored to patient-specific needs also demand robust imaging and modeling tools. Finally, current FDA pathways may not fully accommodate the complexities of such tailored probiotic delivery systems, highlighting the need for updated regulatory frameworks (Jia et al., 2023).

Ethics statement

Ethics approval were waived for this study because no patients' data were reported.

CRediT authorship contribution statement

Felipe Galvez-Jiron: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Lucas de Freitas Pedrosa:** Writing – review & editing, Investigation, Formal analysis. **Luis Silva Lagos:** Writing – review & editing, Conceptualization. **Xin Tang:** Methodology. **Traudy Wandersleben:** Writing – review & editing. **Rodrigo Navia:** Funding acquisition, Writing – review & editing. **Stephan Drusch:** Writing – review & editing, Project administration, Funding acquisition. **Francisca Acevedo:** Writing – review & editing, Supervision. **Paul de Vos:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors disclose no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fhfh.2025.100218.

Data availability

No data was used for the research described in the article.

References

Ahmad, R., Sorrell, M. F., Batra, S. K., Dhawan, P., & Singh, A. B. (2017). Gut permeability and mucosal inflammation: Bad, good or context dependent. *Mucosal Immunology, 10*(2), 307–317. https://doi.org/10.1038/mi.2016.128

Bădăluță, V. A., Curuțiu, C., Dițu, L. M., Holban, A. M., & Lazăr, V. (2024). Probiotics in wound healing. *International Journal of Molecular Sciences, 25*(11), 5723. https://doi.org/10.3390/ijms25115723

Bao, L., Shi, B., & Shi, Y. B. (2020). Intestinal homeostasis: a communication between life and death. *Cell & Bioscience, 10*, 66. https://doi.org/10.1186/s13578-020-00429-9

Barzegar, H., Alizadeh Behbahani, B., Mirzaei, A., & Ghodsi Sheikhjan, M. (2023). Assessing the protection mechanisms against Enterobacter aerogenes by analyzing aggregation, adherence, antagonistic activity, and safety properties of potentially probiotic strain Lactobacillus brevis G145. *Microbial Pathogenesis, 181*, Article 106175. https://doi.org/10.1016/j.micpath.2023.106175

Beukema, M., Jermendi, É., van den Berg, M. A., Faas, M. M., Schols, H. A., & de Vos, P. (2021). The impact of the level and distribution of methyl-esters of pectins on TLR2-1 dependent anti-inflammatory responses. *Carbohydrate Polymers, 251*, Article 117093. https://doi.org/10.1016/j.carbpol.2020.117093

Bostancı, N. S., Büyüksungur, S., Hasirci, N., & Tezcaner, A. (2022). pH responsive release of curcumin from photocrosslinked pectin/gelatin hydrogel wound dressings. *Biomaterials Advances, 134*, Article 112717. https://doi.org/10.1016/j.msec.2022.112717

Brazil, J. C., Quiros, M., Nusrat, A., & Parkos, C. A. (2019). Innate immune cell-epithelial crosstalk during wound repair. *The Journal of Clinical Investigation, 129*(8), 2983–2993. https://doi.org/10.1172/JCI124618

Bugarski, B., Li, Q., Goosen, M. F. A., Poncelet, D., Neufeld, R. J., & Vunjak, G. (1994). Electrostatic droplet generation: Mechanism of polymer droplet formation. *AIChE Journal, 40*(6), 1026–1031.

Celus, M., Kyomugasho, C., Van Loey, A. M., Grauwet, T., & Hendrickx, M. E. (2018). Influence of pectin structural properties on interactions with divalent cations and its associated functionalities. *Comprehensive Reviews in Food Science and Food Safety, 17*(6), 1576–1594. https://doi.org/10.1111/1541-4337.12394

Chelakkot, C., Ghim, J., & Ryu, S. H. (2018). Mechanisms regulating intestinal barrier integrity and its pathological implications. *Experimental & Molecular Medicine, 50*(8), 1–9. https://doi.org/10.1038/s12276-018-0126-x

Cristofori, F., Dargenio, V. N., Dargenio, C., Miniello, V. L., Barone, M., & Francavilla, R. (2021). Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: A door to the body. *Frontiers in Immunology, 12*, Article 578386. https://doi.org/10.3389/fimmu.2021.578386

Dignass, A. U., & Podolsky, D. K. (1993). Cytokine modulation of intestinal epithelial cell restitution: Central role of transforming growth factor beta. *Gastroenterology, 105*(5), 1323–1332. https://doi.org/10.1016/0016-5085(93)90136-z

Dise, R. S., Frey, M. R., Whitehead, R. H., & Polk, D. B. (2008). Epidermal growth factor stimulates Rac activation through Src and phosphatidylinositol 3-kinase to promote colonic epithelial cell migration. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 294*(1), G276–G285. https://doi.org/10.1152/ajpgi.00340.2007

Elshahed, M. S., Miron, A., Aprotosoaie, A. C., & Farag, M. A. (2021). Pectin in diet: Interactions with the human microbiome, role in gut homeostasis, and nutrient-drug interactions. *Carbohydrate Polymers, 255*, Article 117388. https://doi.org/10.1016/j.carbpol.2020.117388

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., & Williams, P. A (2008). Binding behavior of calcium to polyuronates: Comparison of pectin with alginate. *Carbohydrate Polymers, 72*, 334–341. https://doi.org/10.1016/j.carbpol.2007.08.021

Fujiie, S., Hieshima, K., Izawa, D., Nakayama, T., Fujisawa, R., Ohyanagi, H., & Yoshie, O. (2001). Proinflammatory cytokines induce liver and activation-regulated chemokine/macrophage inflammatory protein-3alpha/CCL20 in mucosal epithelial cells through NF-kappaB [correction of NK-kappaB]. *International Immunology, 13*(10), 1255–1263. https://doi.org/10.1093/intimm/13.10.1255

Furue, K., Ito, T., Tsuji, G., Nakahara, T., & Furue, M. (2020). Scratch wound-induced CXCL8 upregulation is EGFR-dependent in keratinocytes. *Journal of Dermatological Science, 99*(3), 209–212. https://doi.org/10.1016/j.jdermsci.2020.07.002

Gall, L., Duckworth, C., Jardi, F., Lammens, L., Parker, A., Bianco, A., Kimko, H., Pritchard, D. M., & Pin, C. (2023). Homeostasis, injury, and recovery dynamics at multiple scales in a self-organizing mouse intestinal crypt. *eLife, 12*, Article e85478. https://doi.org/10.7554/eLife.85478

Galvez-Jiron, F., Tang, X., Gasaly, N., Poncelet, D., Wandersleben, T., Drusch, S., Acevedo, F., & de Vos, P. (2025). Pectin-based encapsulation systems for the protection of beneficial bacterial species and impact on intestinal barrier function in vitro. *Food Hydrocolloids, 160*, Article 110765. https://doi.org/10.1016/j.foodhyd.2024.110765

Grada, A., Otero-Vinas, M., Prieto-Castrillo, F., Obagi, Z., & Falanga, V. (2017). Research techniques made simple: Analysis of collective cell migration using the wound healing assay. *The Journal of Investigative Dermatology, 137*(2), e11–e16. https://doi.org/10.1016/j.jid.2016.11.020

Gyawali, I., Zhou, G., Xu, G., Li, G., Wang, Y., Zeng, Y., Li, J., Zhou, J., Zhu, C., Shu, G., & Jiang, Q. (2023). Supplementation of microencapsulated probiotics modulates gut health and intestinal microbiota. *Food Science & Nutrition, 11*(8), 4547–4561. https://doi.org/10.1002/fsn3.3414

Hou, Q., Ye, L., Liu, H., Huang, L., Yang, Q., Turner, J. R., & Yu, Q. (2018). Lactobacillus accelerates ISCs regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. *Cell Death and Differentiation, 25*(9), 1657–1670. https://doi.org/10.1038/s41418-018-0070-2

Huelsz-Prince, G., Kok, R. N. U., Goos, Y., Bruens, L., Zheng, X., Ellenbroek, S., Van Rheenen, J., Tans, S., & van Zon, J. S. (2022). Mother cells control daughter cell proliferation in intestinal organoids to minimize proliferation fluctuations. *eLife, 11*, Article e80682. https://doi.org/10.7554/eLife.80682

Iizuka, M., & Konno, S. (2011). Wound healing of intestinal epithelial cells. *World Journal of Gastroenterology, 17*(17), 2161–2171. https://doi.org/10.3748/wjg.v17.i17.2161

Jhundoo, H. D., Siefen, T., Liang, A., Schmidt, C., Lokhnauth, J., Béduneau, A., Pellequer, Y., Larsen, C. C., & Lamprecht, A. (2020). Anti-inflammatory activity of chitosan and 5-amino salicylic acid combinations in experimental colitis. *Pharmaceutics, 12*(11), 1038. https://doi.org/10.3390/pharmaceutics12111038

Jia, Z., Xu, X., Zhu, D., & Zheng, Y. (2023). Design, printing, and engineering of regenerative biomaterials for personalized bone healthcare. *Progress in Materials Science, 134*, Article 101072. https://doi.org/10.1016/j.pmatsci.2023.101072

Jindal, M., Kumar, V., Rana, V., & Tiwary, A. K. (2013). An insight into the properties of Aegle marmelos pectin-chitosan cross-linked films. *International Journal of Biological Macromolecules, 52*, 77–84. https://doi.org/10.1016/j.ijbiomac.2012.10.020

Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S. C., Ferrante, M., Shen, B., Bernstein, C. N., Danese, S., Peyrin-Biroulet, L., & Hibi, T. (2020). Ulcerative colitis. *Nature Reviews. Disease Primers, 6*(1), 74. https://doi.org/10.1038/s41572-020-0205-x

Kocaağa, B., Öztürk, Y., Kurçin, H., Zeynep Güner-Yılmaz, Ö., Kurkcuoglu, O., Tatlier, M., Özdemir, İ., Kervancioglu Demirci, E., Kotil, T., Solakoğlu, S., Aksu, B., Batirel, S., Bal-Öztürk, A., & Seniha Güner, F. (2024). Developing multifunctional pectin-based hydrogel for wound dressing: In silico, in vitro and in vivo evaluation. *European Polymer Journal, 216*, Article 113280. https://doi.org/10.1016/j.eurpolymj.2024.113280

Kowalonek, J. (2017). Studies of chitosan/pectin complexes exposed to UV radiation. *International Journal of Biological Macromolecules, 103*, 515–524. https://doi.org/10.1016/j.ijbiomac.2017.05.081

Kwon, J. H., Keates, S., Bassani, L., Mayer, L. F., & Keates, A. C. (2002). Colonic epithelial cells are a major site of macrophage inflammatory protein 3alpha (MIP-3alpha) production in normal colon and inflammatory bowel disease. *Gut, 51*(6), 818–826. https://doi.org/10.1136/gut.51.6.818

Li, C., Peng, K., Xiao, S., Long, Y., & Yu, Q. (2023). The role of Lactobacillus in inflammatory bowel disease: From actualities to prospects. *Cell Death Discovery, 9*(1), 361. https://doi.org/10.1038/s41420-023-01666-w

Liu, Q., Nobaek, S., Adawi, D., Mao, Y., Wang, M., Molin, G., Ekelund, M., & Jeppsson, B. (2001). Administration of Lactobacillus plantarum 299v reduces side-effects of external radiation on colon anastomotic healing in an experimental model. *Colorectal Disease: The Official Journal of the Association of Coloproctology of Great Britain and Ireland, 3*(4), 245–252. https://doi.org/10.1046/j.1463-1318.2001.00244.x

Madali-Kafes, B., Parlak-Yetisen, L., & Dikmen, D. (2025). Exploring the anti-inflammatory effects of microencapsulated probiotic bacteria: In vivo and in vitro evaluation in healthy mouse models. *Journal of the Science of Food and Agriculture, 105*(7), 3753–3759. https://doi.org/10.1002/jsfa.14162

Martinotti, S., & Ranzato, E. (2020). Scratch wound healing assay. *Methods in Molecular Biology,, 2109*, 225–229. https://doi.org/10.1007/7651_2019_259

Masheghati, F., Asgharzadeh, M. R., Jafari, A., Masoudi, N., & Maleki-Kakelar, H. (2024). The role of gut microbiota and probiotics in preventing, treating, and boosting the immune system in colorectal cancer. *Life Sciences, 344*, 122529. https://doi.org/10.1016/j.lfs.2024.122529

McCully, M. L., Kouzeli, A., & Moser, B. (2018). Peripheral tissue chemokines: Homeostatic control of immune surveillance T cells. *Trends in Immunology, 39*(9), 734–747. https://doi.org/10.1016/j.it.2018.06.003

Michon, C., Chapuis, C., Langendorff, V., Boulenguer, P., & Cuvelier, G. (2004). Strain-hardening properties of physical weak gels of biopolymers. *Food Hydrocolloids, 18*(6), 999–1005. https://doi.org/10.1016/j.foodhyd.2004.04.005

Morales-Medina, R., Drusch, S., Acevedo, F., Castro-Alvarez, A., Benie, A., Poncelet, D., Dragosavac, M. M., Defain Tesoriero, M. V., Löwenstein, P., Yonaha, V., Iturralde, R., Gauna Peter, R., & de Vos, P. (2022). Structure, controlled release mechanisms and health benefits of pectins as an encapsulation material for bioactive food components. *Food & Function, 13*(21), 10870–10881. https://doi.org/10.1039/d2fo00350c

Moyer, R. A., Wendt, M. K., Johanesen, P. A., Turner, J. R., & Dwinell, M. B. (2007). Rho activation regulates CXCL12 chemokine stimulated actin rearrangement and restitution in model intestinal epithelia. *Laboratory Investigation; A Journal of Technical Methods and Pathology, 87*(8), 807–817. https://doi.org/10.1038/labinvest.3700595

Nandi, B., Del Valle, J. P., Samur, M. K., Gibbons, A. J., Prabhala, R. H., Munshi, N. C., & Gold, J. S. (2021). CCL20 induces colorectal cancer neoplastic epithelial cell proliferation, migration, and further CCL20 production through autocrine HGF-c-Met and MSP-MSPR signaling pathways. *Oncotarget, 12*(24), 2323–2337. https://doi.org/10.18632/oncotarget.28131

Okamoto, R., & Watanabe, M. (2016). Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease. *Journal of Gastroenterology, 51*(1), 11–21. https://doi.org/10.1007/s00535-015-1098-4

Okumura, R., & Takeda, K. (2017). Roles of intestinal epithelial cells in the maintenance of gut homeostasis. *Experimental & Molecular Medicine, 49*(5), e338. https://doi.org/10.1038/emm.2017.20

Peñalva, R., Martínez-López, A. L., Gamazo, C., Gonzalez-Navarro, C., González-Ferrero, C., Virto-Resano, R., Brotons-Canto, A., Vitas, A., Collantes, M., Peñuelas, I., & Irache, J. (2023). Encapsulation of Lactobacillus plantarum in casein-chitosan microparticles facilitates the arrival to the colon and develops an immunomodulatory effect. *Food Hydrocolloids, 136*, Article 108213. https://doi.org/10.1016/j.foodhyd.2022.108213

Qiu, X., Yin, F., Du, C., Ma, J., & Gan, S. (2024). Alginate oligosaccharide alleviates lipopolysaccharide-induced apoptosis and inflammatory response of rumen epithelial cells through NF-κB signaling pathway. *Animals, 14*(9), 1298. https://doi.org/10.3390/ani14091298

Ramachandran, A., Madesh, M., & Balasubramanian, K. A. (2000). Apoptosis in the intestinal epithelium: Its relevance in normal and pathophysiological conditions. *Journal of Gastroenterology and Hepatology, 15*(2), 109–120. https://doi.org/10.1046/j.1440-1746.2000.02059.x

Rezvanian, M., Ahmad, N., Mohd Amin, M. C., & Ng, S. F. (2017). Optimization, characterization, and in vitro assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. *International Journal of Biological Macromolecules, 97*, 131–140. https://doi.org/10.1016/j.ijbiomac.2016.12.079

Rosales, T. K. O., & Fabi, J. P. (2023). Pectin-based nanoencapsulation strategy to improve the bioavailability of bioactive compounds. *International Journal of Biological Macromolecules, 229*, 11–21. https://doi.org/10.1016/j.ijbiomac.2022.12.292

Said, N., Ibukunoluwa, F., & Won, Y. (2023). Pectin hydrogels: Gel-forming behaviors, mechanisms, and food applications. *Gels, 9*, 732. https://doi.org/10.3390/gels9090732, 9,.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods, 9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Sommer, K., Wiendl, M., Müller, T. M., Heidbreder, K., Voskens, C., Neurath, M. F., & Zundler, S. (2021). Intestinal mucosal wound healing and barrier integrity in IBD-crosstalk and trafficking of cellular players. *Frontiers in Medicine, 8*, Article 643973. https://doi.org/10.3389/fmed.2021.643973

Song, K., Hao, Y., Liu, Y., Cao, R., Zhang, X., He, S., Wen, J., Zheng, W., Wang, L., & Zhang, Y. (2023). Preparation of pectin-chitosan hydrogels based on bioadhesive-design micelle to prompt bacterial infection wound healing. *Carbohydrate Polymers, 300*, Article 120272. https://doi.org/10.1016/j.carbpol.2022.120272

Sturm, A., & Dignass, A. U. (2008). Epithelial restitution and wound healing in inflammatory bowel disease. *World Journal of Gastroenterology, 14*(3), 348–353. https://doi.org/10.3748/wjg.14.348

Sturm, A., Baumgart, D. C., d'Heureuse, J. H., Hotz, A., Wiedenmann, B., & Dignass, A. U. (2005). CXCL8 modulates human intestinal epithelial cells through a CXCR1 dependent pathway. *Cytokine, 29*(1), 42–48. https://doi.org/10.1016/j.cyto.2004.09.007

Suarez-Arnedo, A., Torres Figueroa, F., Clavijo, C., Arbeláez, P., Cruz, J. C., & Muñoz-Camargo, C. (2020). An image J plugin for the high throughput image analysis of in

vitro scratch wound healing assays. *PLoS One, 15*(7), Article e0232565. https://doi.org/10.1371/journal.pone.0232565

Sun, R., Lv, Z., Wang, Y., Gu, Y., Sun, Y., Zeng, X., Gao, Z., Zhao, X., Yuan, Y., & Yue, T. (2024). Preparation and characterization of pectin-alginate-based microbeads reinforced by nano montmorillonite filler for probiotics encapsulation: Improving viability and colonic colonization. *International Journal of Biological Macromolecules, 264*(Pt 1), Article 130543. https://doi.org/10.1016/j.ijbiomac.2024.130543

Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., & Sriamornsak, P. (2007). Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer. *European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V, 67*(1), 132–140. https://doi.org/10.1016/j.ejpb.2007.01.010

Villablanca, E. J., Selin, K., & Hedin, C. R. H. (2022). Mechanisms of mucosal healing: Treating inflammatory bowel disease without immunosuppression?. Nature reviews. *Gastroenterology & Hepatology, 19*(8), 493–507. https://doi.org/10.1038/s41575-022-00604-y

Wang, X., Cao, Z., Zhang, M., Meng, L., Ming, Z., & Liu, J. (2020). Bioinspired oral delivery of gut microbiota by self-coating with biofilms. *Science Advances, 6*(26), eabb1952. https://doi.org/10.1126/sciadv.abb1952

Xin, H., Cai, Z., Hao, J., An, J., Li, Y., Wen, M., & Jia, Z. (2025). Macro/microgel-encapsulated, biofilm-armored living probiotic platform for regenerating bacteria-infected diabetic wounds. *Advanced Healthcare Materials, 14*(7), Article e2403476. https://doi.org/10.1002/adhm.202403476

Xu, C., Ban, Q., Wang, W., Hou, J., & Jiang, Z. (2022). Novel nano-encapsulated probiotic agents: Encapsulate materials, delivery, and encapsulation systems. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 349*, 184–205. https://doi.org/10.1016/j.jconrel.2022.06.061

Xue, X., & Falcon, D. M. (2019). The role of immune cells and cytokines in intestinal wound healing. *International Journal of Molecular Sciences, 20*(23), 6097. https://doi.org/10.3390/ijms20236097

Yan, F., & Polk, D. B. (2020). Probiotics and probiotic-derived functional factors-mechanistic insights into applications for intestinal homeostasis. *Frontiers in Immunology, 11*, 1428. https://doi.org/10.3389/fimmu.2020.01428

Yue, M., Chen, M. M., Zhang, B., Wang, Y., Li, P., & Zhao, Y. (2024). The functional roles of chemokines and chemokine receptors in colorectal cancer progression. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie, 170*, Article 116040. https://doi.org/10.1016/j.biopha.2023.116040

Zare, D., Aryaee, H., Mirdamadi, S., & Shirkhan, F. (2024). The benefits and applications of Lactobacillus plantarum in food and health: A narrative review. *Iranian Journal of Public Health, 53*(10), 2201–2213. https://doi.org/10.18502/ijph.v53i10.16698