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Restoring Mucosal Barrier Function and Modifying Macrophage Phenotype with an Extracellular Matrix Hydrogel: Potential Therapy for Ulcerative Colitis

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Abstract

Background and Aims: Despite advances in therapeutic options, more than half of all patients with ulcerative colitis [UC] do not achieve long-term remission, many require colectomy, and the disease still has a marked negative impact on quality of life. Extracellular matrix [ECM] bioscaffolds facilitate the functional repair of many soft tissues by mechanisms that include mitigation of pro-inflammatory macrophage phenotype and mobilization of endogenous stem/progenitor cells. The aim of the present study was to determine if an ECM hydrogel therapy could influence outcomes in an inducible rodent model of UC.

Methods: The dextran sodium sulphate [DSS]-colitis model was used in male Sprague Dawley rats. Animals were treated via enema with an ECM hydrogel and the severity of colitis was determined by clinical and histological criteria. Lamina propria cells were isolated and the production of inflammatory mediators was quantified. Mucosal permeability was assessed in vivo by administering TRITC-dextran and in vitro using transepithelial electrical resistance [TEER].

Results: ECM hydrogel therapy accelerated healing and improved outcome. The hydrogel was adhesive to colonic tissue, which allowed for targeted delivery of the therapy, and resulted in a reduction in clinical and histological signs of disease. ECM hydrogel facilitated functional improvement of colonic epithelial barrier function and the resolution of the pro-inflammatory state of tissue macrophages.

Conclusions: The present study shows that a non-surgical and non-pharmacological ECM-based therapy can abate DSS-colitis not by immunosuppression but by promoting phenotypic change in local macrophage phenotype and rapid replacement of the colonic mucosal barrier.

Key Words: Extracellular matrix; macrophage activation; barrier function

1. Introduction

Ulcerative colitis [UC] is one of the most common forms of inflammatory bowel disease, and represents a significant global health problem.[1] Since the 1930s, the fundamental approach to treatment has been pharmacological [e.g. 5-amino salicylic acid, immunosuppressive therapy] and/or surgical intervention [e.g. colectomy]. Nearly

a century later, the basic tenets of patient care remain unchanged despite inadequate and less than acceptable results. Each year more than 50% of UC patients suffer from active flares and associated systemic effects. Overall, greater than 20% of patients diagnosed with UC will eventually require radical tissue resection [i.e. colectomy]—an alarming incidence that has remained unchanged over the past 50 years.[2]

UC is a chronic relapsing disease consisting of acute flares followed by periods of remission and healing.[3] Active disease is characterized by chronic inflammation of the colon and defects in intestinal epithelial cell [IEC] barrier function.[4] Based upon observations that bioscaffolds composed of extracellular matrix [ECM] were shown to mitigate inflammation and support functional reconstruction of tissues including the gastrointestinal tract,[5–8] we hypothesized that a similar approach to UC therapy will: [i] abate inflammatory flares not by immune suppression but rather by promoting alternative activation of the local immune cell population; and [ii] induce rapid restoration of the colonic mucosal barrier function not by simply providing a physical barrier between colonic submucosa and luminal contents but rather by promoting proliferation and replacement of the colonic mucosal epithelium. This two-pronged approach was tested by local delivery [enema] of an ECM hydrogel in a rodent model of UC.

The common feature associated with the successful clinical application of ECM bioscaffolds has been modulation of the innate immune response via embedded signalling molecules. Intact and solubilized/hydrogel forms of ECM have been shown to facilitate a rapid and dramatic transition away from an M1-like, pro-inflammatory macrophage phenotype toward an M2-like, pro-healing/regulatory macrophage phenotype.[9,10] Simultaneously, naturally occurring cryptic peptide motifs released or exposed during in vivo degradation of the ECM material, combined with the secreted products of ECM-exposed alternatively activated macrophages, promote stem/progenitor cell chemotaxis, proliferation and differentiation.[11,12] The objective of the present study was to determine if the above-mentioned ECM-induced biological effects could influence outcomes in an inducible rodent model of UC.

2. Materials and Methods

2.1. Experimental design

Ulcerative colitis was induced in male Sprague Dawley rats and treated with a daily enema of ECMH or vehicle [pepsin buffer] only for 7 days to determine the effect of an extracellular matrix hydrogel [ECMH] on colonic inflammation and barrier function. Animals were sacrificed at 7 days and 14 days post-DSS to evaluate the temporal response [n = 14 per time point per treatment] as shown in Supplementary Figure 1, available as Supplementary data at *ECCO-JCC* online. Healthy control rats, which did not receive DSS, were included for comparison at both 7 and 14 days [n = 6 per time point]. The study endpoints included clinical response, histological scores of colon pathology, characterization of the inflammatory response, and barrier function. The effect of ECMH on cell phenotype and epithelial barrier function was also measured in vitro with lamina propria mononuclear cells [LPMCs] and intestinal epithelial cells, respectively.

2.2. ECM hydrogel preparation and formulations

ECM composed of porcine small intestinal submucosa [SIS] was prepared according to a standard protocol.[13] In brief, porcine small intestine was harvested immediately following euthanasia, rinsed of contents in de-ionized water, and frozen. The tissue was thawed and the tunica mucosa, the tunica serosa, and tunica muscularis externa were mechanically removed, leaving behind the tunica submucosa and basilar portions of the tunica mucosa [termed SIS]. Decellularization of the SIS material was conducted by rinsing in de-ionized water for 2472 h before treatment with 0.1% peracetic acid/4% ethanol and subsequent saline and water rinses. SIS-ECM was frozen, lyophilized, and comminuted with a Wiley Mill using a #60-mesh screen, and digested at 10 mg/ml dry weight with 1 mg/ml pepsin [Sigma, St Louis, MO] in 0.01N HCl while stirring for 20–26 h at 21–23°C. Digest was stored in aliquots at -20°C and pH neutralized with 0.1M NaOH before use. Hydrogel formation was induced by the neutralization step and an accompanying temperature increase to approximately 37°C following administration of the enema. All in vivo studies used an ECM hydrogel [ECMH] concentration of 8 mg/ml and all in vitro studies used an ECMH concentration of 500 µg/ml. The choice of 8 mg/ml ECMH concentration was based on a hydrogel concentration that supported robust gel formation within 10 min of placement within the colon, would adhere to the ulcerated colonic wall for greater than 12 h, and did not exhibit time-dependent viscosity as was observed in the 12 mg/ml ECMH [Supplementary Figure 2, available as Supplementary data at *ECCO-JCC* online]. To examine the effects of ECMH on cells in vitro, the ECMH concentration had to be reduced to prevent gelation within the cell culture and 500 µg/ml was used based on previous studies.[10,14]

^{14}C-labeled ECMH was prepared as stated above with the intestines of pigs that were injected with ^{14}C-tagged proline, as previously described.[15] Fluorescein isothiocyanate [FITC]-labelled ECMH was prepared with a protein labelling kit [Thermo PierceNet] per manufacturer's instructions.[11]

2.3. ECMH adhesion testing

The muco-adhesion strength of ECMH was measured using a modified detachment force technique.[16] A uni-axial tensile testing machine [MTS Insight; MTS Systems, Eden Prairie, MN] equipped with a 10-N load cell was used for all tensile strength measurements. Two colon sections were glued to steel washers [diameter 12.7 mm] with mucosa facing outward and one washer was glued to the bottom of a 24-well plate [diameter 15.6 mm]. The ECMH was prepared by neutralizing with 1/10 volume of 0.1M NaOH and 1/9 volume of 10x PBS; then 0.5 ml of ECMH was added onto the bottom tissue, and the top tissue was added and allowed to penetrate into the gel to a predetermined depth before incubating at 37°C for 1 h. After incubation, the upper washer was slowly withdrawn at a constant speed of 5 mm/min until a failure occurred between the surfaces.

2.4. Animals and husbandry

All procedures and animal studies were approved and conducted in compliance with the University of Pittsburgh Radiation Safety Committee and the Institutional Animal Care and Use Committee. Male Sprague Dawley rats, 8–12 weeks of age, were obtained from standard vendor [Harlan] and were housed and environmentally acclimatised for 7–10 days. Animals were housed in standard laboratory conditions with a temperature of 21–23°C and 12-h dark/light cycles. Rats were allowed ad libitum access to food and water throughout the study.

2.5. Disease induction and monitoring

Dextran sulphate sodium [DSS] salt 5% [36 000–50 000 MW; MP Biomedical] was prepared daily in de-ionized water and administered to rats by ad libitum drinking for 7 days and the animals were monitored daily. Animal weight and consumption of food and water

were tracked for each animal. Disease activity [i.e. stool consistency, presence of blood in stool, and weight loss] was measured every other day [i.e. Days 1, 3, 5, 7 etc.] and scored on a range of 0 to 4. Stool was scored for consistency [0 = normal, 2 = loose, 4 = diarrhoea] and presence of blood [0 = none, 2 = occult, 4 = gross bleeding]. Stool was tested for the presence of blood using ColoScreen ES Lab Pack Fecal Occult Tests. Weight loss compared with baseline was scored as follows: 0 = none, 1 = 1–5%, 2 = 5–10%, 3 = 10–20%, and 4 = > 20%.

2.6. ECMH retention studies with FITC- and ^{14}C-ECMH

To determine hydrogel retention time, rats were administered FITC-labelled or ^{14}C-labelled ECMH via enema following disease induction. Eighteen rats were divided into two groups based on ECMH formulation [FITC- and ^{14}C-ECMH] and sacrificed at 2 h, 12 h, and 24 h post enema [n = 3 per time point per ECMH formulation]. Explanted colons from FITC-ECMH-treated rats were processed to be optically clear such that the luminal contents were visible by fluorescent imaging. Immediately following sacrifice, all samples were protected from light to prevent photo-bleaching of the FITC conjugate. Optical clearing of the colons was initiated by incubating in Dent's fixative [1:4 dimethyl sulphoxide [DMSO]: acetone] for 2 h. Colons were then permeabilized and bleached in Dent's bleach [1:4:1 DMSO: acetone: H_2O_2] for 1 h. Optically cleared colons were then imaged on a Fluorescent gel imager [Chemidoc Touch, Biorad]. Exposure time was set to a control sample of FITC-ECMH and kept constant for all subsequent images.

For ^{14}C measurements, the entire colon of each rat was individually flash-frozen in liquid nitrogen and homogenized. The frozen tissue was ground with mortar and pestle and mixed until homogeneous. Approximately 40 mg of tissue samples was analysed by accelerated mass spectrometry [AMS]. Non-treated controls were used to subtract the background ^{14}C levels in native tissue.

2.7. Explanting and scoring of colonic tissue

Animals were sacrificed at predetermined time points as described previously. Euthanasia was achieved by CO_2 inhalation and subsequent cervical dislocation in accordance with the guidelines of the American Veterinary Medical Association [AVMA]. Following euthanasia, the colon was resected following a ventral abdominal midline incision. A continuous colon segment was collected, spanning from the rectum to the caecum, and photographed. Colon length was measured as an indicator of disease activity. The colon was opened longitudinally and assessed grossly by investigators blinded to the treatment group for damage according to the metrics outlined in Supplementary Table 1.

The distal region of colon, 9 cm in length, was cut into thirds and opened longitudinally. Specimens were then collected for histological examination, ex vivo organ culture, and myeloperoxidase measurement. The colon specimens were paraffin-embedded and tissue sections [5 µm] that were obtained from 2 to 8 cm from distal to proximal colon were stained with haematoxylin and eosin [H&E] for representative histological scoring. The distal and proximal tissue sections were separated onto two slides and histological scoring was performed according to Table 1 by six blinded investigators.

2.8. TRITC-dextran permeability assay

Colonic mucosal permeability was assessed by enteral administration of TRITC-dextran [molecular mass 4.4 kDa; Sigma]. Rats were administered TRITC-dextran [1 ml, 10 mg/ml] enema 4 h before sacrifice. Whole blood was obtained at the time of sacrifice in serum collection tubes and allowed to clot undisturbed at room temperature for at least 30 min. The clot was removed by centrifuging at 1000g for 10 min at 4°C. TRITC-dextran concentration in the serum were determined in triplicate on a SpectraMax plate reader [Molecular Devices], with serial dilutions of TRITC-dextran used as a standard curve.

Table 1. Criteria for histological scoring of colon specimens.

Feature	Score	Description
Inflammation extent	0	No inflammation
	1	Mild inflammation in mucosa
	2	Moderate-severe inflammation in mucosa
	3	Mild inflammation into the submucosa
	4	Moderate-severe inflammation into the submucosa
Ulceration	0	0%
	1	1–25%
	2	26–50%
	3	51–75%
	4	76–100%

2.9. Organ cultures

Full-thickness biopsies were obtained following sacrifice from the explanted colon of each experimental and control animal at Day 7 and Day 14 using a 3-mm dermal punch as described previously.[17] Tissue specimens were cultured at 37°C with 5% CO_2 for 48 h. The supernatants were then harvested and stored at -80°C until the amount of tumour necrosis factor alpha [TNFα] and prostaglandin E2 [PGE2] was measured using enzyme-linked immunosorbent assays [ELISA assays].

2.10. LPMC isolation and culture

Lamina propria mononuclear cells [LPMCs] were isolated from rats following colitis induction with DSS as described above. The colon was explanted, cleared of mesenteric fat tissue, and regions of Peyer's patches were excised. The colon was then split in half longitudinally, cut into pieces, and dissociated into single-cell suspensions using a lamina propria dissociation kit [Miltenyi] according to manufacturer's instructions. The suspension was then separated along a 40/70% Percoll gradient. The cells were suspended in RPMI 1640 containing 10% fetal bovine serum [FBS] and 100 U/ml penicillin and streptomycin, and then placed in 96-well plates at 2×10^5 cells per well with or without the addition of 500 µg/ml ECMH or vehicle [i.e. pepsin buffer]. After 48 h of incubation, the supernatant was collected and stored at -80°C until assayed for TNFα and PGE2 with ELISAs.

2.11. IEC culture

For in vitro barrier function assays, IECs [Caco-2, passages 24–28, ATCC] were cultured to approximately 80% confluence in MEM containing non-essential amino acids, 1mM sodium pyruvate, and 20% FBS. The functional response of IECs to ECMH was evaluated using rapid differentiation system [Corning Biocoat HTS Caco-2 Assay] per manufacturer's instructions. Confluent and differentiated cell monolayers were challenged with 100 ng/ml lipopolysaccharide [LPS] for 2 h and then treated with ECMH for 48 h.

2.12. Transepithelial electrical resistance [TEER] measurement

TEER of Caco-2 monolayers was measured with an Epithelial Voltohmmeter [EVOM2, World Precision Instruments]. Before seeding Caco-2 cells, electrical resistance of the supporting filter and buffer medium was measured and subtracted from the total

electrical resistance determined with the monolayer to calculate the TEER of the monolayer. Only differentiated monolayers with TEER values greater than 300 $\Omega \times cm^2$ were used in the study.

2.13. Immunolabelling

To determine the macrophage response following ECMH treatment, paraffin-embedded histological sections were deparaffinized and immunolabelled for a pan-macrophage marker [CD68] and indicators of the M1-like [TNFα] and M2-like [CD206] macrophage phenotypes. All primary antibodies were confirmed to cross-react with rat epitopes. For visualizing the presence of Caco-2 adhesion proteins, trans-well inserts were fixed in 4% paraformaldehyde and immunolabelled for epithelial cadherin [E-cad]. Sections were imaged at five random fields per tissue section. Quantification of localized staining was achieved using a custom image analysis algorithm developed using CellProfiler Image Analysis Software.

2.14. Statistical analysis

The sample size used in the present study was determined based on a power analysis using pilot study data in combination with previously published relevant studies. All animals were numbered and randomly assigned to a treatment. All investigators responsible for scoring were blinded to the experimental groupings. Quantitative outcomes were compared with a one-way or two-way analysis of variance [ANOVA] and post hoc Tukey test to determine differences between groups. All statistical analysis was performed using SPSS Statistical Analysis Software [SPSS, IBM]. Data are reported as mean ± standard deviation [SD] unless otherwise stated.

3. Results

3.1. ECMH is adhesive to colon tissue

The therapeutic efficacy of ECMH is reliant upon its ability to adhere to the colon wall and interface with the resident cells. ECMH has the distinctive property of reverse thermal gelation and the hydrogel properties are dependent upon material characteristics [Supplementary Figure 3, available as Supplementary data at *ECCO-JCC* online]. Results of adhesion testing show that ECMH is muco-adhesive, with a dose-dependent increase in adhesion strength when tested on healthy colon [Figure 1A]. Importantly, the 8 mg/ml ECMH dose used in the present study maintains equivalent adhesion strength in colitic rat colon when compared with healthy tissue [Figure 1B]. It is noteworthy that mucosal adherence is not simply a property of thermos-reversible gels. For example, Pluronic F-127 [20%; Sigma] did not show adhesion strength greater than the negative control [data not shown]. When delivered via enema to colitic rats, the residence time of the ECMH is greater than 24 hours; 2 h after administering the enema, about 50% of the ^{14}C-ECMH remains attached to the colon wall and approximately 10% of the initial ECMH enema remained after 24 h [Figure 1C]. These results were corroborated by visualization of FITC-ECMH [Figure 1D]. Together the results show that ECMH material properties at 8 mg/ml allow injection as a liquid, and the subsequent rapid gelation ensures that the treatment remains localized for at least 24 h. Based on these data, a daily enema treatment was used to measure the therapeutic efficacy of ECMH.

3.2. ECMH treatment mitigates disease state

The DSS experimental model is a well-accepted UC-like self-limiting colitis phenotype with epithelial barrier defects.[18] Clinical signs of colitis [e.g. weight loss, stool blood, and stool consistency] were present following 3 days of exposure to 5% DSS in drinking water and reach their peak at 6 days [Supplementary Figure 4, available as Supplementary data at *ECCO-JCC* online]. ECMH treatment diminished clinical symptoms of UC in this rodent model. ECMH-treated animals did not lose as much weight [at Days 1 and 3] and had less blood in stool [at Days 3 and 5] compared with the vehicle

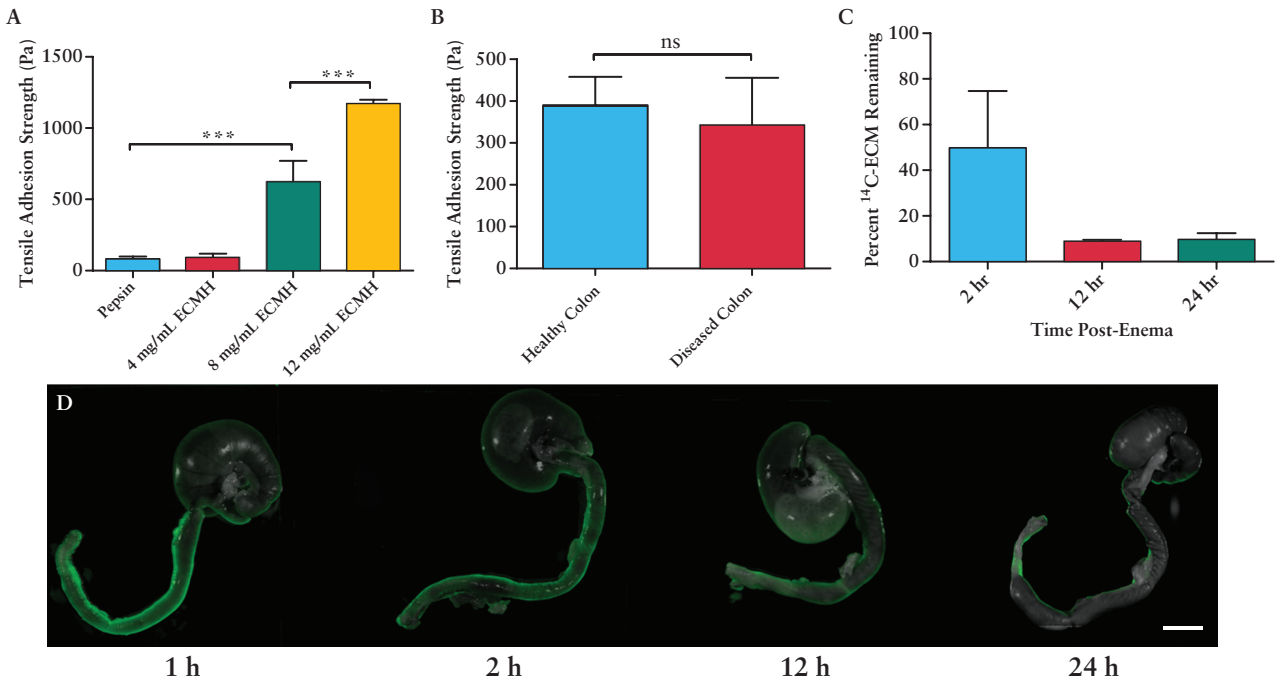

Figure 1. ECMH is mucoadhesive. Tensile tests show dose-dependent increase in adhesion strength of ECMH to healthy colon [A] and equivalent adhesion in healthy vs diseased colon [B]. The resident time of ECMH following enema delivery was tested with ^{14}C [C] and FITC-labelled ECMH [D]. Scale bar = 1 cm. ECMH, extracellular matrix hydrogel.

control. The shortening of the colon that was present at Day 0 [Supplementary Figure 3D] was no longer evident by Days 7 and 14 across all groups [Figure 2D]. ECMH treatment resulted in a reduction in the gross score compared with the vehicle at Day 7 [Figure 2E]. Histomorphological analysis also showed that ECMH is therapeutic in the present model and resulted in diminished signs of inflammation and a lower degree of ulceration at 7 and 14 days in both distal and proximal tissue sections [Figure 3; and Supplementary Figure 5, available as Supplementary data at *ECCO-JCC* online].

3.3. ECMH restores epithelial barrier function

A defect in gut barrier function and increased permeability can lead to inflammatory bowel disease even in the presence of an intact immune system.[19] Results of the TRITC-dextran permeability assay showed that the barrier function of ECMH-treated animals is similar to that of healthy animals at 7 days, whereas the colonic epithelial barrier in the vehicle-treated control group remain impaired compared with the healthy control [Figure 4A]. Differentiated and LPS-damaged monolayers of IECs respond to ECMH treatment in vitro, with functional recovery as shown by TEER readings [Figure 4B]. The increased barrier function was associated with an increased presence of E-cadherin, one of the most important cell-cell adhesion proteins in the gut. ECMH treatment leads to approximately 50% increase in E-cadherin positive cells compared with negative controls [Figure 4C, D].

3.4. ECMH mitigates the inflammatory response

Recognized inflammatory mediators of IBD [i.e. TNFα and PGE2] were measured in the present study. LPMCs isolated from colitic rats were plated and exposed to ECMH. The ECMH treatment resulted in a substantial reduction in the production of TNFα [Figure 5A] by the LPMCs but had no effect on PGE2 production [Figure 5B]. Organ cultures of biopsies collected from rats following ECMH or vehicle control treatment showed that secreted PGE2 was similar to healthy controls in the ECMH-treated animals, whereas the vehicle controls had significantly elevated levels of mucosal PGE2 [Figure 5C]. Secreted levels of TNFα were below detection in the organ cultures regardless of experimental condition at the time points studied.

The effect of ECMH on macrophage phenotype in DSS-colitis was evaluated by quantifying the number of $CD68^+$ macrophages in the colon that co-express TNFα or CD206. Interestingly, the absolute number of individually labelled $CD68^+$, $CD206^+$, and $TNF\alpha^+$ cells was the same across all treatment groups [data not shown], but ECMH treatment resulted in a reduction in the number of co-labelled $CD68^+/TNF\alpha^+$ inflammatory macrophages at Day 7 [Figure 5D]. The fact that ECMH did not affect the amount of global $TNF\alpha^+$ cells but did reduce the number of $CD68^+/TNF\alpha^+$ cells suggests a direct role for ECMH in modulating the macrophage response by reducing the number of inflammatory macrophages present in the colonic tissue.

4. Discussion

The present study shows that an ECM hydrogel composed of ECM mitigates the pro-inflammatory macrophage phenotype and restores barrier function in a rodent model of UC. It is noteworthy that the total number of macrophages was not changed by ECMH treatment, but rather the phenotype of this cell population was changed. In addition, the barrier function was not restored by the physical presence of the hydrogel but rather by the restoration of an effective mucosal epithelium. These effects are a distinct departure from the immunosuppressive [defensive] and surgical [salvage] methods currently used to treat UC in humans. More than half of all patients with UC do not achieve long-term remission and many require colectomy.

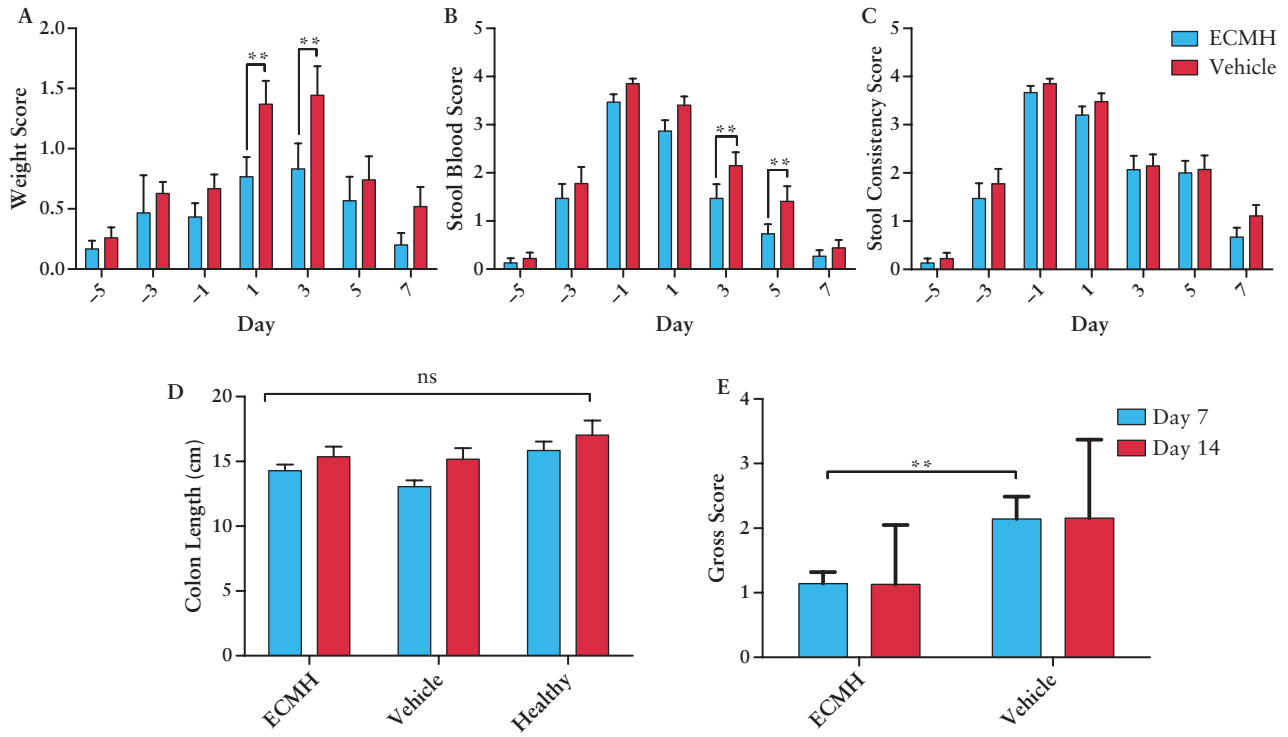

Figure 2. ECMH treatment reduces disease activity. The effect of ECMH treatment on clinical symptoms [AC], on colon length [D] and on gross score at explant [E] was tracked and compared with the vehicle [pepsin] alone. ECMH, extracellular matrix hydrogel.

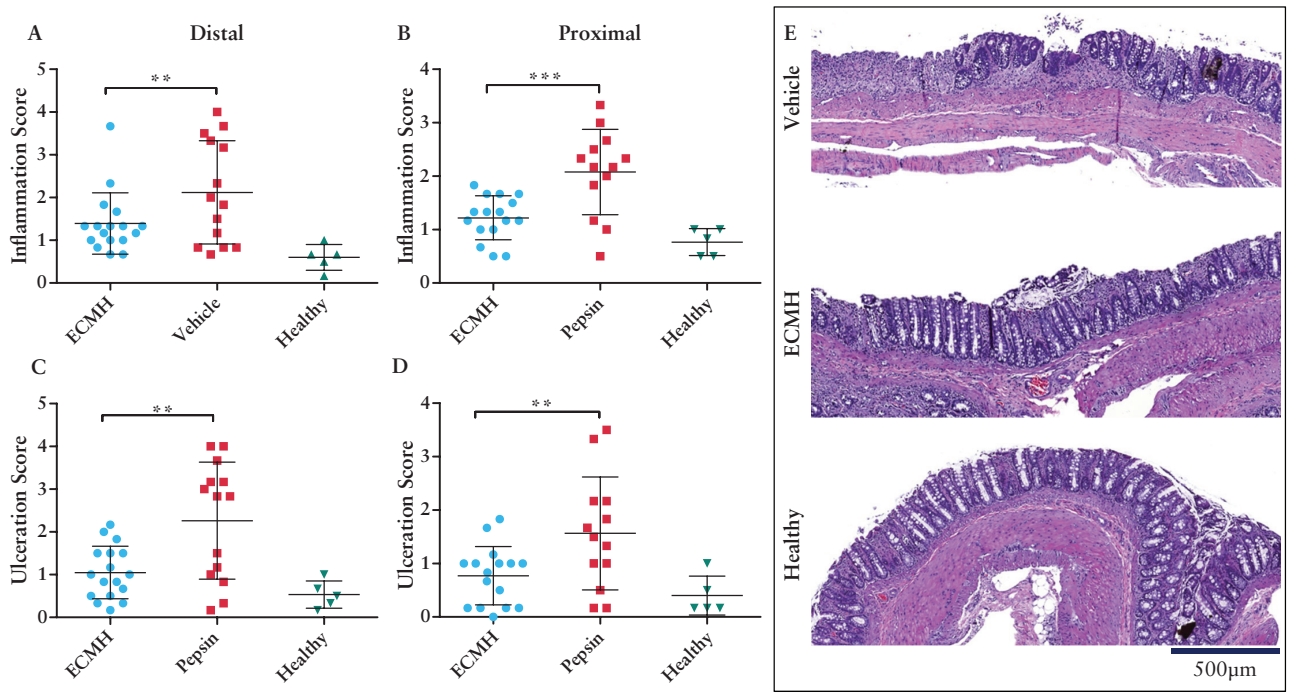

Figure 3. ECMH treatment lowers histological score. Distal and proximal tissue sections stained with haematoxylin and eosin were scored by blinded investigators and compared with vehicle/pepsin buffer. The extent of inflammation [A, B] and degree of ulceration [C, D] were quantified at 7 days. Representative images used for inflammation scoring are shown in panel E. ECMH, extracellular matrix hydrogel.

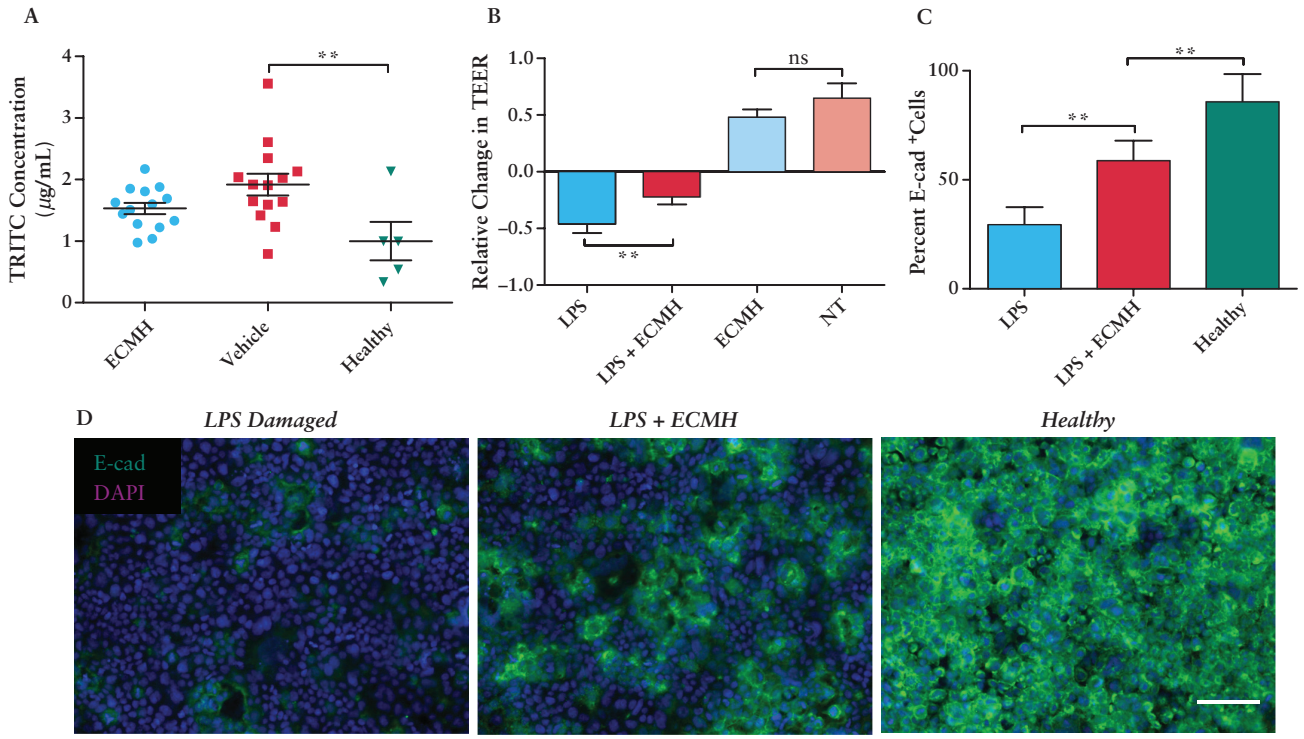

Figure 4. ECM restores barrier function. TRITC-dextran permeability assay showed that the barrier function of ECMH-treated animals was similar to that of healthy animals, whereas the colonic epithelial barrier in the vehicle-treated control group remained impaired compared with the healthy control [A]. Differentiated and LPS-damaged monolayers of IECs responded to ECMH treatment in vitro with functional recovery as shown by TEER readings [B]. The increased barrier function was associated with an increased presence of E-cadherin compared with negative controls [CD]. Scale bar = 100 μm. ECMH, extracellular matrix hydrogel; LPS, lipopolysaccharide; IECs, intestinal epithelial cells; TEER, transepithelial electrical resistance.

UC is a complex immune-mediated disease characterized by diffuse inflammation confined to the mucosa and submucosa of the colon and rectum. The inflammatory infiltrate consists of neutrophils, lymphocytes, and macrophages that penetrate the epithelium and lead to ulceration and crypt abscessation.[20] Macrophages, dependent upon their phenotype, are important effector cells of both the

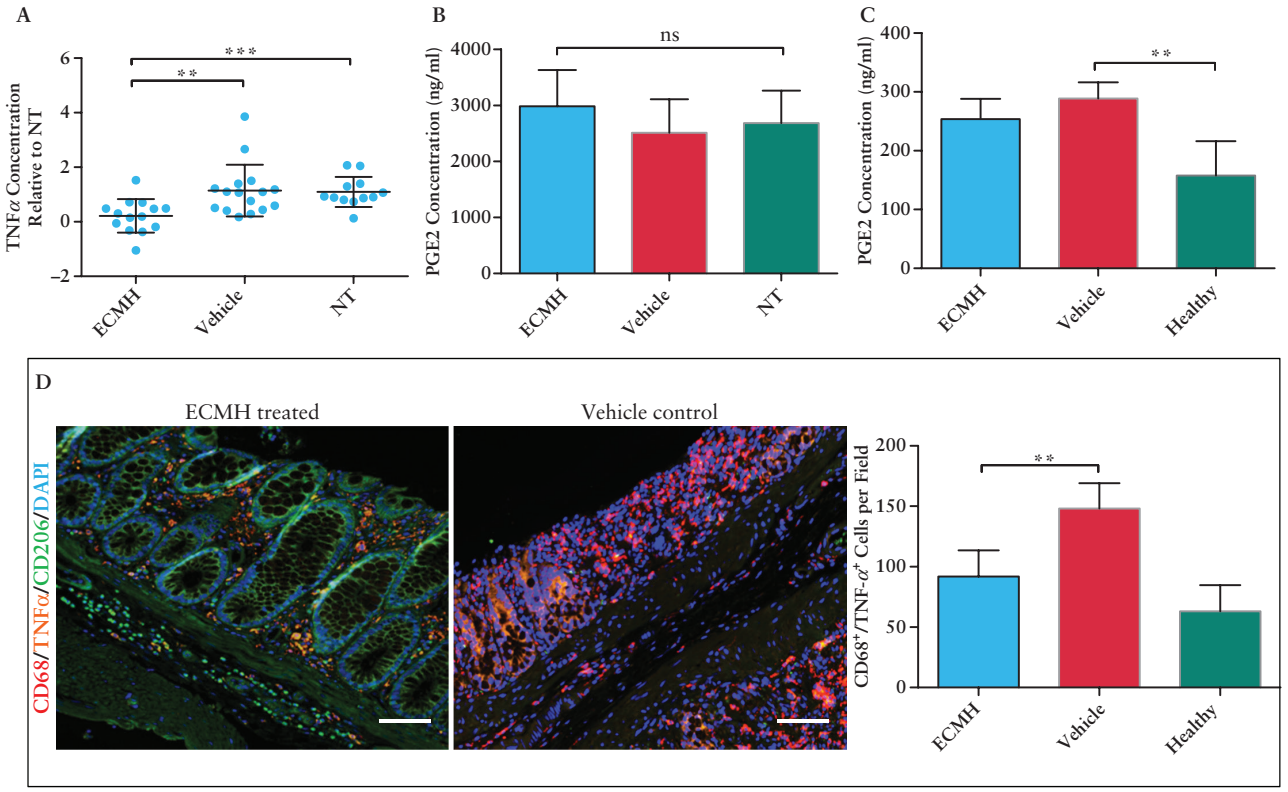

Figure 5. ECMH mediates inflammation. Exposure of lamina propria cells to ECMH results in lowered levels of TNFα [A] but didn't affect PGE2 production [B]. Organ cultures, however showed ECMH had a significant impact on PGE2 levels [C]. ECMH treatment led to a decrease in total number of M1, TNFα-expressing macrophages [D]. Scale bar = 100 μm. ECMH, extracellular matrix hydrogel; TNFα, tumour necrosis factor alpha; PGE2, prostaglandin E2.

initiation/maintenance of inflammatory response and the resolution/regeneration processes. Regulatory M2-like macrophages have been associated with mucosal healing outcomes[21,22] in preclinical models of IBD. Similarly, the restoration of functional tissues via ECM bioscaffold-mediated events in a variety of regenerative medicine applications has been shown to be either associated with, or dependent upon, a timely shift in macrophage phenotype toward M2 predominance.[23–25]

The findings of the present study are consistent with, and analogous to, the known mechanisms by which ECM-based approaches facilitate the constructive remodelling of injured tissues in other anatomical locations.[5,26–30] Specifically, ECM materials derived via decellularization of a variety of allogeneic or xenogeneic source tissues have been shown to induce a phenotypic transition from the pro-inflammatory macrophage and lymphocyte phenotype toward a regulatory, anti-inflammatory and healing phenotype,[9,31–33] and to promote endogenous stem/progenitor cell activation and recruitment.[11,29,34,35] Furthermore, the findings in the present study are similar to the results in patients with oesophageal adenocarcinoma who were subjected to complete surgical removal of long-segment oesophageal mucosa and placement of a solid [i.e. not hydrogel] form of an ECM bioscaffold. These patients showed a rapid restoration of the oesophageal mucosa and oesophageal function without recalcitrant stricture.

The combination of promoting a shift in macrophage function from inflammation to wound healing and facilitating the restoration of an intact colonic mucosa is a distinct departure from current therapeutic strategies for UC, which are focused upon immune suppression [e.g. corticosteroids and anti-TNFα compounds] with the associated local and systemic effects. Immune suppression is clearly different from maintaining complete functionality of the immune system while redirecting its biological objectives. In fact, a robust immune system is necessary for a healthy, functional gastrointestinal tract.[36–39]

The use of immunosuppressive and/or anti-inflammatory compounds has a limited ability to facilitate mucosal healing,[40,41] and yet the disrupted mucosal barrier integrity is a key component in the pathogenesis of UC. Barrier dysfunction enables the ingress of luminal antigens and pathogens, and the continuous activation of a pro-inflammatory immune response in the lamina propria and the associated chronic inflammation that is the hallmark of UC. Restoration of barrier function is therefore an important therapeutic target in UC. In the present study, ECMH therapy had a protective downstream effect on the epithelial cells of the colonic mucosa. Results show that ECMH facilitated functional improvement of the epithelial barrier function and suggest that ECMH acts therapeutically either by limiting epithelial cell damage and/or by actively promoting mucosal integrity.

Recognition that effector cells of the immune system, such as the macrophages and T helper cells, not only promote classic inflammatory processes but also orchestrate the temporal inhibition of inflammation and initiation of functional tissue remodeling,[25,31,42–45] provides the opportunity to re-examine immunosuppressive strategies for treatment of diseases such as UC. Although the signalling molecules that influence macrophage and lymphocyte phenotype transition are not fully understood, there is suggestive evidence that at least some of these regulators reside within the ECM.[9,10,25] Results of the present study suggest that ECMH modulates the innate immune response not by directly promoting an M2-like macrophage phenotype but rather by reducing the number of TNFα-expressing

M1-like pro-inflammatory macrophages, thus shifting the micro-environmental milieu from inflammation to repair. It is noteworthy that M2-like macrophages are connected to UC-associated carcinogenesis, and a sustained M2-like phenotype may not be optimal for UC therapies. However, the tumour-associated macrophage [TAM] M2-like phenotype differs from the regulatory M2-like phenotype associated with tissue restoration.[46,47] The present study showed the ECMH-mediated macrophage response to be transient and associated with a reduction of TNFα, a suspected participant in inflammation-mediated colon carcinogenesis.[48,49]

Limitations of the present study include the use of one animal model and only two surface markers for macrophage phenotype. The DSS-colitis model was chosen because the model features innate immunity and epithelial barrier defects that are central to the present study's hypothesis. The outcomes in the DSS-colitis model can be effective in predicting clinical treatment of IBD.[18] It is well established that when DSS administration is halted, the animals will begin to spontaneously recover[50] and therefore differences among groups require examination of the temporal response. Only two markers for macrophage phenotype were used for immunolabelling, and we chose the most representative marker of UC-like inflammation [TNFα] and for M2-like macrophages [CD206]. Macrophages are a heterogeneous and plastic cell population, and the use of a single marker to delineate phenotype can result in ambiguity. TNFα, in particular, was chosen for the present study because of its integral role in the pathogenesis of UC[51]; however, it is logical and plausible that the use of other markers could provide additional insight into the effects of ECMH treatment upon the resident macrophage population in the colitic micro-environment.

Despite limitations, the present study shows that ECMH restores epithelial barrier function and modulates macrophage phenotype away from a pro-inflammatory state. Two physiological processes, the colonic barrier function and pro-inflammatory response, were positively influenced by ECMH therapy. Given the muco-adhesive properties of ECMH, it would also be feasible to use ECMH as a carrier for local delivery of pharmocologicals. One benefit of ECMH, in addition to the therapeutic efficacy, is that the well-accepted safety profile of ECM products may allow for accelerated transition to the clinic.

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Conflict of Interest

The authors have no financial or competing obligations to declare.

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Author Contributions

TJK and SFB developed the study concept and design. TJK, JD, ES, AS, AC, NT, and LW contributed to data acquisition. TJK, JD, and SFB analysed and interpreted data. All authors critically reviewed and approved the final version of the manuscript.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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