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# Effect of a matrix metalloproteinase sequestering biomaterial on Caco-2 epithelial cell barrier integrity in vitro

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**Abstract**

A novel matrix metalloproteinase sequestering biomaterial (MI Theramer™ beads) restored the epithelial barrier in a double chamber in vitro test system after disruption by Cytochalasin D and the secretion of the metalloproteinase MMP-2. MI beads are chemically modified (hydroxamated) poly(methacrylic acid-co-methyl methacrylate). We are exploring the utility of this material in inflammatory bowel disease (IBD), in which one manifestation is a compromised intestinal epithelial barrier. In a first step towards this goal we incubated MI beads (or polymethyl methacrylate control beads) with Caco-2 epithelial cells and mesenchymal 3T3 fibroblasts on two sides of a Matrigel®-coated filter and used Cytochalasin D (Cyto D) to activate MMP-2 (secreted by the 3T3 cells), disrupt actin filaments of Caco-2 cells and render the epithelial barrier leaky, as measured by dextran fluorescein equilibration. Addition of MI beads to Cyto D-treated cells inhibited active MMP-2 and prevented equilibration of dextran fluorescein. This study is the first step in showing a potential benefit to local (as opposed to systemic) inhibition of metalloproteinases in IBD or other intestinal inflammatory diseases.


*Keywords:* MI Theramer™ biomaterial beads; Matrix metalloproteinases; Epithelial barrier; Actin cytoskeleton

## 1. Introduction

Metalloproteinases (MMPs) are a family of structurally related zinc-containing proteinases. They are implicated in processes such as cell migration, metastasis and infiltration; cytokine activation; and tissue remodeling, damage and repair [1–3], and are the most prominent proteinases implicated in inflammation. In the digestive tract, MMPs have been reported to be involved in chronic and acute inflammatory diseases such as inflammatory bowel diseases (IBDs) and enterobacteria-induced inflammation (e.g. *Helicobacter pylori*, *Salmonella typhimurium*, *Vibrio cholerae*), and in animal models of experimental bowel inflammation. The importance of the epithelial barrier in disease predisposition is supported by the finding of abnormal intestinal permeability in some first-degree relatives with Crohn's disease [4]. Chronic IBDs, such as Crohn's disease and ulcerative colitis, are commonly classified as autoimmune diseases. Their most common symptoms are mild to severe diarrhea and abdominal pain. Severe diarrhea can lead to dehydration, a drop in blood pressure, a rapid heartbeat and continuous blood loss in the stool, which can result in anemia. Treatment of IBD is symptomatic, using anti-inflammatory drugs and immunosuppressive agents. In severe cases, surgery is indicated, although an effort is made to avoid surgery because of the recurrent nature of the disease. New approaches are sought to reduce IBD symptoms.

Given the evidence implicating MMPs in gastrointestinal inflammation, effecting a reduction in MMP activity associated with gastrointestinal diseases has a promising therapeutic potential. Indeed, a few encouraging studies have been published, where treatments with systemic MMP inhibitors (MMPIs) reduced tissue injury and inflammation in animal models of IBD [5–8]. However,

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the systemic MMPIs used in these studies have the potential to affect MMPs throughout the organism, yielding severe MMP anti-target side effects [9]; such side effects have been a major limitation to the use of these drugs in other contexts [10–12]. For example, utilizing MMPIs in cancer has not been successful in clinical trials and the complexity involved has been elucidated [13]. A partial explanation for this failure is the wide range of action and abundance of MMPs: some MMPIs can have beneficial target effects while others augment the disease by affecting anti-targets, counterbalancing its therapeutic effect. This has led to a drive to create more specific MMPIs.

On the other hand, arguments have been made for using weaker drugs [9] but delivering them locally. Delivering MMPIs to the site of the disease could offer a real improvement in treatment, by reducing the need for high systemic concentrations of the drug in order to achieve effective levels of the drug in the vicinity of the target, thereby reducing potential side effects in non-target organs. Recently, a new biomaterial-based approach for treatment of MMP-related diseases has been introduced: the MI Theramer™ (Rimon Therapeutics Ltd., Toronto, Ont.). This material, in the form of small diameter (~200 μm) beads, contains an MMP sequestering hydroxamate group on a polymer poly(methacrylic acid-co methyl methacrylate) backbone [14a]. The MI beads are administered locally and, because they are non-soluble, there are no systemic consequences. In a first-in-humans pilot (32 patient) clinical trial it has reduced MMP activity in chronic wound fluid exudate and dramatically improved wound bed quality and the healing trajectory [14b].

Both host and bacterial proteinases are implicated in the loss of epithelial barrier permeability, and the inhibition of proteinases enhances barrier integrity [15–18]. In vitro, double chamber culture systems are one way of demonstrating the influence of mesenchymal cells proteinases on epithelial barrier integrity [15]. For example, it was shown that disruption of the actin cytoskeleton with Cytochalasin D (Cyto D) enhances MMP expression and activation [19–21] by enhancing expression of the membrane-associated MMP activator MT1-MMP [22]. In this communication, we demonstrate in an in vitro double chamber system that MI beads restore Caco-2 colon adenocarcinoma epithelial cell barrier integrity, previously disrupted by activation of MMP with Cyto D, via inhibition of active MMP-2. We suggest that MI beads may offer a promising biomaterial therapeutic alternative for inflammatory gastrointestinal diseases such as Crohn's disease and ulcerative colitis.

## 2. Materials and methods

### 2.1. Materials

All chemicals were obtained from Sigma (Oakville, Ont., Canada) unless otherwise indicated. MI Theramer™ beads (~200 μm in diameter) were supplied by Rimon Therapeutics, Ltd. (Toronto, Ont., Canada). The MI Theramer™ is a hydroxamate-containing material that was produced from crosslinked poly(methylmethacrylate-co-methacrylic acid) microspheres by treatment via a mixed anhydride intermediate, as described elsewhere [14a]. Approximately 20% of the surface methacrylic acid groups were converted to hydroxamic acid. Beads were also endotoxin-free [14a]. Poly(methyl methacrylate) beads (PMMA; Polysciences Inc., Warrington, PA) of the same size served as control material.

### 2.2. Cell culture in the double chamber system

Caco-2 colorectal adenocarcinoma epithelial cells and 3T3 cells were obtained from ATCC (Manassas, VA; CRL-2102, CRL-1658, respectively) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and penicillin/streptomycin (Invitrogen, Burlington, ON; 100 units: 100 mg $ml^{-1}$, respectively). Cells were plated and maintained in a double chamber system (Millicell-PCF inserts, 12 mm diameter, 0.4 μm pore size; Millipore Corporation, Billerica, MA) as previously described [24] with modifications. Briefly, 150 μL aliquot of ice-cold, 1:3 diluted Matrigel (BD Biosciences, Mississauga, ON, Canada), was applied onto the lower face of reversed Millicell inserts placed in 24-well culture plates. Excess Matrigel was removed. Matrigel on inserts was allowed to gel by incubation at 37 °C for 30 min. 3T3 cells ($2 \times 10^5$/150 μl) were plated on the reverse side of the chamber (Fig. 1A) and allowed to attach to the substrate for 3 h at 37 °C. Caco-2 cells were plated in the upper chamber at a density of $4 \times 10^5$ cells/400 μl. Six hundred microliters of growth medium was added to the lower chamber, and the cells were incubated overnight. The next day, the chambers were transferred to new 24-well plates and washed thoroughly three times with serum-free DMEM. Treatments (beads or Cyto D as well as dextran fluorescein (Invitrogen 3000 MW)) were added to the lower chambers. The next day, conditioned medium was collected for MMP-2 zymography or dextran fluorescein analysis (see below).

### 2.3. Morphology of cells in the double chamber system

Twenty-four hours after plating, cells were treated with 0.5 μg $ml^{-1}$ Cyto D for an additional 24 h. Cells were washed three times with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 10 min and washed a further three times with PBS. The filters were then removed from the Millicell assembly and embedded in paraffin, before thin sections were stained with hematoxylin–eosin.

### 2.4. Preparation of beads and Cyto D treatment

Beads at the desired final density (see below) were suspended in 100% ethanol overnight and washed three times with serum-free DMEM. After the final wash, the pH was

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