Καθιέρωση και χαρακτηρισμός υδρογέλης από αποκυτταρωμένο έντερο ποντικού

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Table of Contents

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INTRODUCTION

The intestinal structure

The human gastro-intestinal (GI) tract has a surface area of 30-40m^2 (Helander and Fändriks 2014). A single epithelial layer covers the intestine, and its main roles are nutrient uptake and protection against pathogens. Beneath the epithelium, the lymphatic system is responsible for the proper distribution of these nutrients to the rest of the organism. Although its large surface area renders the epithelial cells exposed to mechanical stress and other possible threats, such as pathogen intrusion, their short lifetime (3-5 days) (Darwich et al. 2014) diminishes these threats. The intestinal epithelium is organized into crypts and villi. Multiple crypts surround one villus. From the crypts, continuous streams of new cells that differentiate and head towards the tips of the villi are generated (Gehart and Clevers 2019). More particularly, crypt base columnar (CBC) cells divide continuously, in the bases of the crypts, and give rise to mature intestinal epithelial cells. There are six different mature cell types which are separated into absorptive (enterocytes and M cell) and secretory (Paneth, goblet, enteroendocrine and tuft cell) lineages. They are characterized by different roles such as immune regulation, metabolic control and nutrient uptake (Gehart and Clevers 2019).

Intestinal stem cells

Since 1974, crypt base columnar (CBC) cells were introduced as continuously cycling cells at the bottom of the crypts (Cheng and Leblond 1974). Although there was no solid proof that CBC cells are stem cells, a study performed 30 years later provided the necessary information to prove it. More particularly, Barker et al utilized genetic lineage tracing tools to show that Lgr5 is a specific marker for CBC cells and that every mature cell was offspring of this $Lgr5^+$ CBC cell (Barker et al. 2007). Besides CBC cells, "+4" cells have been introduced as another candidate stem cell population. These cells are located between the stem cell and the transit amplifying zones (Figure 1) and their contribution to epithelial regeneration after injury is reported in many studies (Sangiorgi and Capecchi 2008; Takeda et al. 2011; Montgomery et al. 2011). The stem cells in the crypt are well protected from the harsh environment existing in the intestine. The unique architecture of the crypt (Kaiko et al. 2016) in combination with the secretion of mucus from goblet cells close to the crypt opening (Johansson, Larsson, and Hansson 2011) and the production of anti-microbial products from Paneth cells (Porter et al. 2002) isolates and protects the crypts from pathogens and their products. The development of the 3D culture of intestinal organoids led to the understanding of the necessary and sufficient components of the stem cell niche. These include both epithelial (Paneth cells) (Sato et al. 2011) and mesenchymal cells, which contribute to WNT pathway activation, EGF signaling and BMP inhibition.

Paneth cells

These cells belong to the secretory lineage, and they do not follow the common rule of moving upwards during differentiation. They move downwards, in between the CBC cells in the crypt (Potten and Loeffler 1990) (Figure 1). Their direct contact with CBC cells is necessary because Paneth cells secrete WNT ligands, EGF and Notch stimuli for the preservation of stemness (Sato et al. 2011). Moreover, their protective role is of much importance as described above. In an environment where expression of WNT is high and Notch signaling is absent, cells turn into Paneth cells. When the first Paneth cell is formed, surrounding cells maintain their stem cell characteristics, due to expression of Notch ligands (Sancho, Cremona, and Behrens 2015). Moreover FGF signaling plays a crucial role in Paneth cell differentiation, as deletion of this path leads to deterioration of cell numbers (Vidrich et al. 2009).

Goblet cells

Goblet cells constitute the most frequently renewed secretory lineage cell type. Their major role is to secrete mucus and coat the intestinal epithelium in order to protect it from external pathogens. Moreover, this mucus layer creates an environment to allow microbes to live in close proximity to mammalian host cells in mutualistic symbiosis in the steady state, thus contributing to the increase of nutrients obtained from the diet. Recent evidence has uncovered another role of goblet cells, this of transferring luminal substances to Lamina Propria-Antigen Presenting Cells (LP-APCs) through endocytosis. This process is called GAP formation and it takes place in the small intestine and distal colon (McDole et al. 2012; Knoop and Newberry 2018). When Notch signaling is absent and IL-4 and IL-13 are present (by Tuft cells activation), differentiation pathway leads to the formation of goblet cells.

Enteroendocrine cells

Enteroendocrine cells are a diverse group of specialized cells that play key roles in the regulation of gastrointestinal tract physiology. These cells are distributed throughout the epithelial lining of the stomach, small and large intestines (Figure 1) and have the unique ability to detect luminal signals and secrete hormones into the blood. Based on the hormones they produce, enteroendocrine cells can be further divided into subtypes. Different subtypes of enteroendocrine cells exhibit distinct molecular properties and respond to specific stimuli, allowing fine-tuned coordination of digestive processes and metabolic regulation. For example, enteroendocrine cells release hormones such as gastrin, cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1), which regulate gastric acid secretion, appetite and satiety, and glucose metabolism, respectively. These hormones act as chemical messengers, carrying messages to other cells and organs in the body (Worthington, Reimann, and Gribble 2018; Gribble and Reimann 2019).

Tuft cells

These cells are characterized by the presence of microvilli on their apical surfaces, giving them a brush-like appearance, and they play a key role in sensing and responding to environmental stimuli. These cells express the transcription factor POU2F3. Through specific receptors and signaling molecules, tuft cells are able to recognize various luminal stimuli such as microbial products, allergens, and parasites. Upon activation, tuft cells secrete a variety of factors, including cytokines, chemokines, and growth factors that induce immune responses and regulate tissue homeostasis. Their role in regulating type 2 immune responses is well documented, as is their involvement in the response to helminth infection. In addition, recent studies have also shown that tuft cells are involved in the regulation of intestinal stem cell function and epithelial repair after injury (Gerbe et al. 2016; von Moltke et al. 2016; Howitt et al. 2016).

Enterocytes

As the primary cells of the intestinal epithelium, enterocytes play crucial roles in intestinal nutrient absorption, barrier function, and immune regulation (Citi 2018). These specialized cells are responsible for the uptake of nutrients from the gut lumen into the bloodstream, a process facilitated by the expression of various transporters on their apical membrane (Turner 2009). Enterocytes help maintain the integrity of the intestinal barrier by forming tight junctions with neighboring cells that regulate paracellular permeability and prevent the penetration of noxious substances. Regarding immune regulation, enterocytes express pattern recognition receptors (PRRs), which recognize microbial components and elicit an appropriate immune response. This interaction with the gut microbiota helps to establish immune tolerance and maintain gut homeostasis (Peterson and Artis 2014).

M cells

M cells are specialized epithelial cells found in the intestinal wall that play a key role in sampling luminal antigens and eliciting intestinal immune responses (Knoop et al. 2009). These cells are predominantly found in the follicle-associated epithelium of Peyer's patches, an important site of gut-associated lymphoid tissue (Knoop et al. 2009; Mabbott et al. 2013). They lack microvilli and have a high transcytosis capacity, allowing them to transport luminal antigens across the epithelial barrier (Mabbott et al. 2013). M cells deliver the captured antigen to immune cells, such as dendritic cells and lymphocytes within Peyer's patches, triggering an antigen-specific immune response (Knoop et al. 2009). This process is critical for the induction of mucosal immunity and the establishment of tolerance, which is the ability of the immune system to tolerate innocuous food and commensal antigens in response to pathogen threats (Suzuki et al. 2008). The role of M cells in antigen uptake and immune regulation highlights their importance in maintaining intestinal immune homeostasis and defense against pathogens.

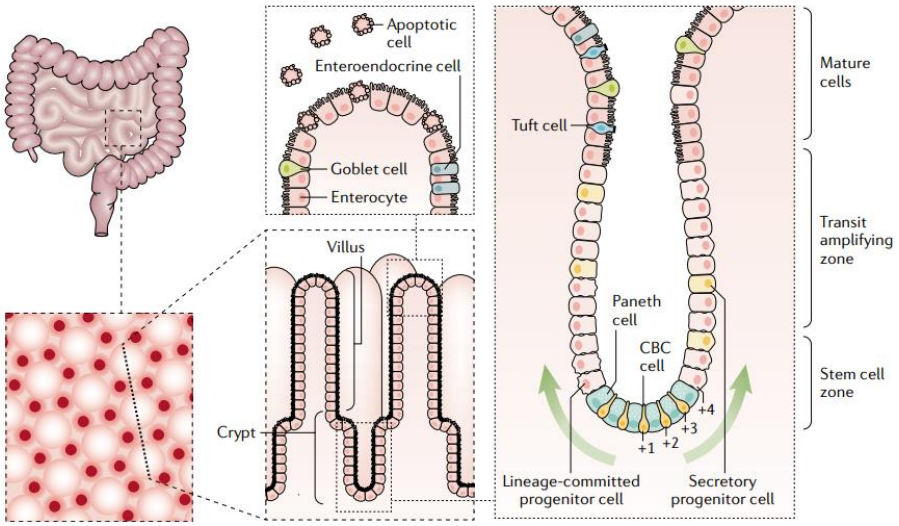

Figure 1: The intestinal epithelium (Adapted from Gehart and Clevers 2019).

Other cells of the intestinal mucosa include mesenchymal, endothelial, and immune cells, with diverse roles in the regulation of immune and epithelial homeostasis and gut functionality. Among them, intestinal mesenchymal cells secrete Wnt and BMP signaling molecules to affect the behavior of intestinal stem cells (ISC) and therefore act at the ISC niche.

Inflammatory Bowel Disease (IBD)

Tissue injury or infection (Figure 2) leads to inflammation, which removes damaged cells and microbes. This is followed by resolution of inflammation and regeneration of the epithelium to restore organ function. Dysregulation of the mechanisms underlying these processes leads to conditions such as chronic inflammation, fibrosis, and cancer. Inflammatory Bowel Disease (IBD) is a chronic condition that affects the digestive tract. The two main types of IBD are Crohn's disease and ulcerative colitis. In both types, the immune system attacks the lining of the digestive tract, causing inflammation and damage. Symptoms can include abdominal pain, diarrhea, rectal bleeding, weight loss, and fatigue (“What Is Inflammatory Bowel Disease (IBD)? | IBD” 2022).

It is well known that the intestinal mucosa, site of very close proximity between foreign antigens (microbes/food) and immune system cells, has evolved defense mechanisms (mucosal checkpoints) to prevent uncontrolled responses of the immune system to bacterial or food antigens

[tolerance/symbiosis/homeostasis]. IBD refers to the failure of one or more mucosal checkpoints, resulting in a dysregulated interaction between the tissue and the microbiota, resulting in a persistent proinflammatory response (Bamias, Jia, and Cominelli 2013). The majority of IBD-associated polymorphisms affect genes that encode for proteins involved in bacterial-immune interactions, such as, NOD2, IL10 and IL23R (Jostins et al. 2012). Moreover, studies have shown that mice with genetic modification of genes of the immune system develop chronic intestinal inflammation (Mizoguchi et al. 2016). Studies from human patients have verified the increased expression levels of various factors of the immune system (Singh et al. 2016). All the current treatments for IBD patients involve the blockade of immunological pathways that lead to improvement of the disease. Such therapies are Anti-TNF, Anti-IL-12/23, Corticosteroids, methotrexate, JAK Inhibitors and Anti-integrin (Cai, Wang, and Li 2021).

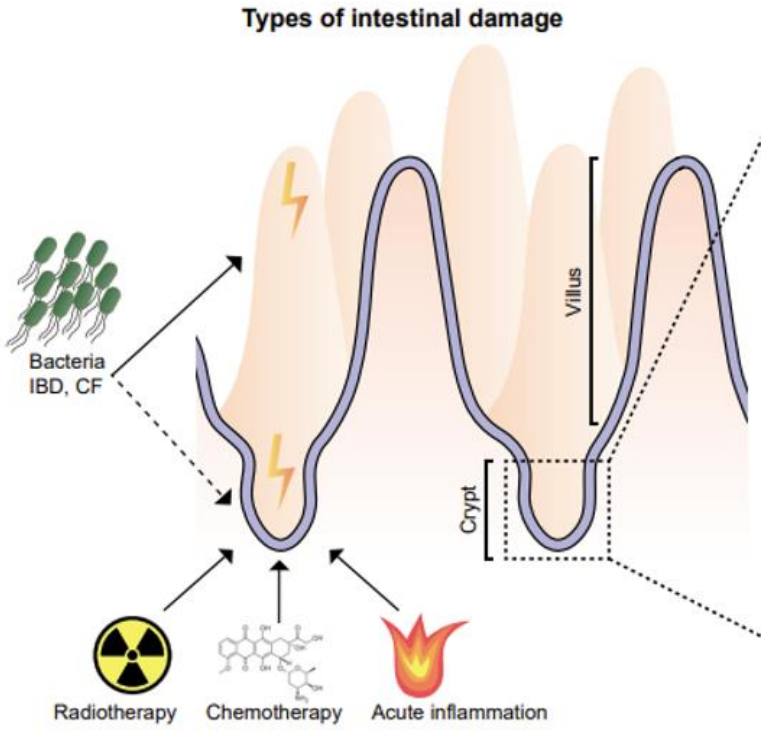

Figure 2: The small intestinal stem cell niche and types of epithelial injuries (Adapted from Hageman et al. 2020)

The Extracellular Matrix (ECM)

The extracellular matrix (ECM) is a dynamic network of macromolecules that surrounds and supports cells in tissues and organs of multicellular organisms (Hynes 2009). It can be divided in two categories based on its location and structure: the interstitial connective tissue and the basement membrane (Bonnans, Chou, and Werb 2014; Theocharis et al. 2016). The ECM is composed of various structural proteins, including collagens, laminins, fibronectins, and elastins, as well as proteoglycans and glycosaminoglycans (Frantz, Stewart, and Weaver 2010). The composition and organization of the ECM are critical for many cellular functions, including cell adhesion, migration, proliferation, differentiation, and survival (Rozario and DeSimone 2010). Moreover, the ECM plays important roles in tissue development, maintenance, and repair, as well as in pathologies such as cancer, fibrosis, and inflammation (Naba et al. 2016).

Collagens are the most abundant proteins in the ECM, providing tensile strength and elasticity to tissues (Karsdal et al. 2017). Laminins and fibronectins contribute to cell adhesion and migration by

interacting with cell surface receptors, such as integrins (Humphries, Byron, and Humphries 2006). Proteoglycans and glycosaminoglycans, including hyaluronan, chondroitin sulfate, and heparan sulfate, regulate the hydration and viscoelastic properties of the ECM, as well as the availability and activity of growth factors and cytokines (Iozzo 1998).

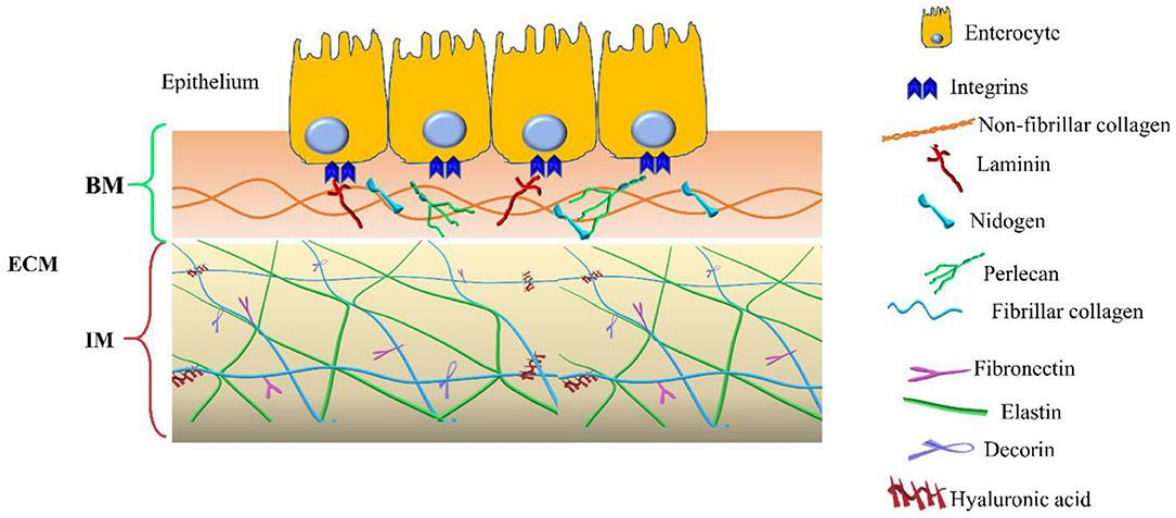

Figure 3: Schematic representation of the main components of the two ECM compartments: basement membrane (BM) and interstitial matrix (IM) (Adapted from Pompili et al. 2021)

Changes in the ECM are the result of multiple remodeling mechanisms, which can be grouped into four major processes: 1) ECM deposition, which alters the abundance and composition of ECM components, thereby affecting ECM biochemical and mechanical properties, 2) post-translational chemical modification of the ECM alters the biochemical and structural properties of the ECM, 3) release of bioactive ECM fragments and ECM-sequestered factors through proteolytic degradation, and 4) force-mediated physical remodeling affects the number of ECM tissue cell migration channels by arranging ECM fibers and openings (Winkler et al. 2020). The ECM undergoes continuous remodeling mediated by various enzymes, such as matrix metalloproteinases (MMPs), and lysyl oxidases (LOXs) (Kessenbrock, Plaks, and Werb 2010; Bonnans, Chou, and Werb 2014). These enzymes cleave, modify, or cross-link ECM proteins, resulting in changes in their mechanical, structural, and biochemical properties. ECM remodeling is critical for tissue repair and regeneration but can also contribute to pathological conditions such as fibrosis and cancer invasion (Bonnans, Chou, and Werb 2014).

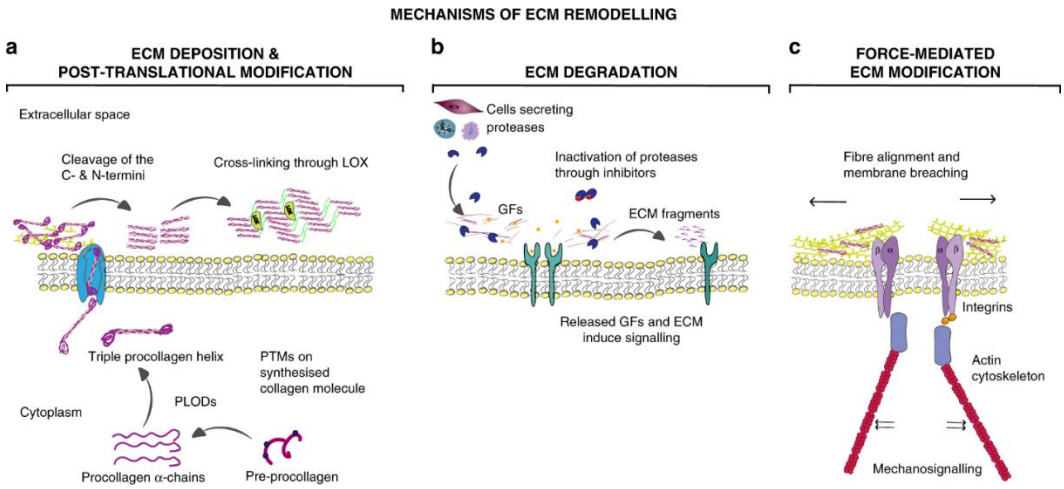

Figure 4: Mechanisms of ECM remodeling (Adapted from Winkler et al. 2020)

The roles of ECM in homeostasis

Regulation of development and stem cell fate

Beginning in the 1980s and early 1990s, the ECM was recognized as a critical regulator of embryogenesis and tissue-specific development. Gene analysis and manipulation studies in model organisms revealed that several developmental defects could be mapped back to ECM proteins (Adams and Watt 1993). The most important cues seem to be the coordinated balance between cell-ECM and cell-cell dynamics. Through cell-to-cell adhesion, the ECM can physically function beyond the first layer of ECM-bound cells and into the tissue/organ interior. As with cell-ECM adhesion, there are multiple types of adhesive contacts between cells in the developing embryo, for example various adherens junctions, desmosomes and tight junctions (Walma and Yamada 2020).

ECM-directed cell migration is also a critical determinant of cell fate specification during development. Embryonic cell migration can include amoeboid, mesenchymal or lobopodial 3D modes of cell migration (Yamada and Sixt 2019). Mesenchymal and lobopodial migration involve extensive integrin-mediated adhesion to surrounding ECM substrates, whereas amoeboid migration can involve non-specific interactions with ECM. Mesenchymal migration is characterized by cells using actin-driven lamellipodial or filopodial protrusions to adhere to, produce force against and migrate in or on the ECM (Caswell and Zech 2018; Plutoni et al. 2019).

The ECM not only regulates stem cell fate and tissue-specific differentiation but also modulates stem cell behavior in adult organisms. Despite the potential for both self-renewal and differentiation, adult stem cells often remain quiescent for long periods of time (L. Li and Clevers 2010). The ECM microenvironment of these adult stem cells, the stem cell niche, is thought to regulate stem cell behavior, including maintaining their long-term dormancy. In fact, ECM is an important part of this niche, providing a microenvironment in which stem cells can remain dormant until receiving stimuli that promote their expansion and differentiation (Gattazzo, Urciuolo, and Bonaldo 2014).

Spatial-mechanical regulation of cell phenotype

Motility, growth, survival, and differentiation of cells are influenced by the ECM, achieved through the binding of cell surface receptors identifying ECM moieties. Recognition by these cellular receptors leads to intracellular signaling and cytoskeletal reorganization, initiated by integrins, discoidin

domain receptors (DDRs), syndecans, CD44, receptor for hyaluronic acid-mediated motility, and Robo receptors which all bind to the ECM (Muncie and Weaver 2018).

Stiffness of the ECM plays a critical role in the structure and function of specific differentiated tissues and also has the power to regulate cellular constituent growth and survival, as well as direct their lineage-specific differentiation and motility. For example, by altering the elasticity of the substrate, human MSCs can seemingly differentiate towards various distinct lineages (Engler et al. 2006). When cells are subjected to a stiff extracellular matrix , the actomyosin tension within the cells increases. This phenomenon appears to have a connection to the cells' ability to activate TGF-β. By growing cells on or within a physically stressed or stiff ECM, this connection can be significantly strengthened (Wipff et al. 2007). A stiff ECM can also, activate mechanosensitive ion channels to induce cell growth and survival, and stimulate cell migration and differentiation. For instance, provided that the stiffness of the ECM reaches a certain level, Piezo transmembrane cation channels can be activated through ECM ligation, and this can effectively regulate cell differentiation. This is achieved by substantially increasing the cellular actomyosin tension. Piezo1 activation through ECM tension was demonstrated by Pathak and team, highlighting how it could lead to calcium-dependent neurogenesis in human neural stem cells, with astrogenesis being hindered by its suppression or removal (Pathak et al. 2014).

Moreover, Yes- associated protein and transcriptional coactivator with PDZ-binding motif (YAP/TAZ) have become a major area of interest as evidence has emerged that their activity can be directly regulated by ECM stiffness(J. Hao et al. 2014). YAP/TAZ are usually transported to the nucleus when they are not phosphorylated. Afterwards, they participate in activating gene expression by partnering with other transcription factors. Confirmation that mechanotransduction is a vital player in YAP/TAZ signaling was presented after cells were cultured on rigid surfaces or encouraged to expand via patterned ECM ligands, resulting in YAP being prompted to move into the nucleus. By contrast, nuclear YAP translocation was prevented when cells were plated on soft substrates or when cell spreading was prevented (Dupont et al. 2011).

Control of the availability and presentation of growth factors and morphogens

Cellular receptors are key in intracellular signaling for modifying gene expression in cell fate and tissue development are regulated via the roles played by members of the TGF-β superfamily, particularly BMPs and TGF-β. By this, cellular and tissue behavior undergo modification (Dijke and Hill 2004). Given their critical role during development and tissue homeostasis, it is not surprising that the storage, release, and activation of TGF-β and BMPs are both tightly regulated by ECM binding(Wipff et al. 2007). In addition, many growth factors involved in wound healing interact with the ECM, including platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF). The ECM is made up of many molecules that are in constant contact with both growth factors and neighboring cells (Schultz and Wysocki 2009; Eckes, Nischt, and Krieg 2010).

Regulation of immune cell mobility, survival and function

For both migrating and tissue resident cells, the ECM helps determine not only their location but also regulates their survival and function. For example, fibronectin and laminin in the ECM interact with integrins on immune cells, thereby promoting cell adhesion and migration (Hynes 2002). In addition, the ECM acts as a reservoir of cytokines and chemokines that activate or recruit immune cells to sites of injury or infection (Page-McCaw, Ewald, and Werb 2007). Conversely, immune cells (or relevant surrounding cells) express distinct metalloproteinases that degrade components of the ECM such as collagen and proteoglycans, to inhibit or promote immune cell migration (McMahon et al. 2021).). ECM dysregulation has been implicated in a variety of immune-mediated diseases, including IBD, rheumatoid arthritis, and cancer (Petrey and de la Motte 2017; Lu, Weaver, and Werb 2012). Overall, the relationship

between the ECM and the immune system is a complex two-way relationship and the ECM plays an important role in the function and regulation of immune cells.

ECM Remodeling in IBD

In the inflamed mucosa of IBD patients, there is an increased deposition of collagens and fibronectin, which are major components of the ECM. Collagens, particularly types I, III, and V, are known to accumulate in the intestinal mucosa during chronic inflammation in IBD. This excessive collagen deposition leads to tissue fibrosis, which can result in structural damage and functional impairment (Rieder et al. 2011). Fibronectin, a glycoprotein involved in cell adhesion and tissue repair, is also elevated in the inflamed mucosa of IBD patients. It plays a role in chronic inflammation and fibrosis by promoting leukocyte recruitment and activation (Rieder et al. 2011). Besides collagens and fibronectin, alterations in the expression of other ECM proteins have been observed in IBD, suggesting their involvement in disease pathogenesis. Laminins show changes in their expression and spatial distribution. These alterations can affect the interactions between the ECM and intestinal epithelial cells, potentially compromising barrier function and tissue homeostasis. Proteoglycans, including syndecans and glycosaminoglycans, also exhibit altered expression in the inflamed mucosa of IBD patients. These changes can impact cell signaling, immune responses, and tissue repair processes (Yamamoto-Furusho et al. 2019)

Influence on Epithelial Barrier Function

The intestinal epithelium is kept intact thanks to the structural support bestowed by the ECM. By establishing a physical scaffold, the ECM aids in anchoring epithelial cells and maintaining their polarity, thus enabling the sealing and stabilization of tight junctions and other intercellular connections (Latella et al. 2014). Increased intestinal permeability in IBD occurs when the epithelial barrier function is compromised due to disruptions in ECM components. Such examples are increased intestinal permeability, low-grade intestinal inflammation, and increased susceptibility to DSS-induced colitis (Laukoetter et al. 2007), increased claudin-2 expression and inflammatory cytokine production (Marchelletta et al.,2021). Such alterations weaken the integrity of epithelial barrier leading to the penetration of luminal antigens, microbial products and bacteria into mucosa. As a consequence, immune responses and inflammation are triggered (Lechuga et al. 2023). While interacting with the immune system and intestinal microbiota, the ECM creates a convoluted web of interrelations that regulate the epithelial barrier function. The gut's glycosaminoglycans and proteoglycans, can either increase or limit microbial attachment and colonization, as they interact with microbial components. These interactions have the ability to reshape the microbiota's composition, and hence could determine barrier function (Rawat et al. 2022). The immune cells that populate the gut's mucosa are also ECM interactors and can alter barrier function by releasing cytokines and other immune mediators (Alam and Neish 2018).

Modulation of Immune Responses

Immune cell behavior and responses can be modified by the ECM components present in the intestinal mucosa. Acting as a physical scaffold, it creates an environment that can manipulate immune cell adhesion, migration, and activation. Additionally, it is able to regulate immune cell recruitment that occurs during IBD related inflammation. Apart from their structural role, certain ECM components like proteoglycans and glycosaminoglycans can interact with immune cells and modify their abilities, this includes cytokine production, antigen presentation and phagocytosis (Tomlin and Piccinini 2018). Many ECM proteins harbour bioactive domains which act as direct ligands on a large number of immune cell receptors (Lebbink et al. 2006; 2009). Immune cell-ECM ligand interactions play a role in both promoting

and inhibiting inflammatory responses, while also contributing to the maintenance of a stable environment and the creation of pathways that lead to disease. For example, collagen is a high affinity ligand for leukocyte associated immune receptor (LAIR)-1 expressed on most immune cells including T cells, B cell, NK cells, monocytes, macrophages, monocyte-derived DCs, mast cells, eosinophils, and basophils (Meyaard 2010). Collagen-LAIR-1 association promotes immune suppressive phenotype essentially holding the immune response in check and contributing to homeostasis. Similarly, differential expression of LAIR-1 by immune cells or interruption of collagen-LAIR-1 binding by the action of soluble receptor LAIR-2 (Meyaard 2010), may be involved in driving disease processes. In addition, newly synthetized or fragmented ECM proteins, generated upon remodeling or during injury, are recognized by immune cells as DAMPs, or in this case, ECM-DAMPs (Bollyky et al. 2009; Frevert et al. 2018). PRRs like TLR2 and TLR4 are crucial in activating immune cells and endothelial cells after engaging with ECM-DAMPs. These molecules are recognized by multiple receptors, leading to immune system activation in cells like monocytes, T cells, and macrophages. The immunomodulatory properties of ECM-DAMPs are largely attributed to the effectors TLR2 and TLR4 (Tu et al. 2008). Within the intricacies of ECM remodeling and degradation processes lie the fragments known as matricryptins. These protein fragments, comprised of collagen and laminin derivations, have been identified as moderators of immune cell activity and cytokine production in the context of IBD. When interacting with immune cell receptors, the matricryptins act as signaling molecules, enabling them to regulate the behavior of these cells. For instance, collagen-derived matricryptin fragments have been observed to affect dendritic cell function, macrophage polarization, and T-cell activation in IBD (de Castro Brás and Frangogiannis 2020).

Fibrosis and ECM Remodeling

The extracellular matrix (ECM) becomes affected by fibrosis due to chronic inflammation in patients with IBD, resulting in excessive deposition and remodeling. Fibronectin, collagens, and proteoglycans are the ECM components that accumulate in tissues during this process. This unfavorable occurrence can be a result of ongoing inflammation and irregular wound healing responses. The consequences of fibrosis include tissue stiffness and distortion of the architectural structure, causing impaired functionality of organs in IBD. The severity of these changes can influence the motility of organs such as the intestines, resulting in bowel obstruction or the formation of strictures in IBD. In addition to these physical changes, fibrosis can also lead to architectural distortions and interrupt normal tissue organization, further worsening organ dysfunction in IBD (Park et al. 2023).

Mesenchymal cells

Mesenchymal cells (MCs) play a crucial role in maintaining tissue homeostasis. Mesenchymal cells, especially fibroblasts, are a group of non-epithelial, non-hematopoietic, non-endothelial, non-neuronal cells that produce growth factors, cytokines, and extracellular matrices for the normal growth and development of multiple organs (Powell et al. 2011). One of their most well-known roles is to provide structural support in almost every organ. This is achieved by their ability to produce ECM and enzymatically remodel it in a spatiotemporal and organ specific manner (Bonnans, Chou, and Werb 2014). Moreover, their contribution in regulating the immunological homeostasis and coordinating the immune responses is fundamental (Perez-Shibayama, Gil-Cruz, and Ludewig 2019). In addition, pericytes and perivascular smooth muscle cells seem to be in charge of adjusting vasoconstriction and normal vascular permeability by interacting with endothelial cells (Armulik, Genové, and Betsholtz 2011). In addition, resident mesenchymal precursors are present in various organs, such as the gut and skeletal muscle, and make important contributions, especially during tissue repair (Sacchetti et al. 2016). Beyond

their multipotent characteristics, MCs are of much importance in preserving the stem cell pool in specific niches by secreting Wnts and BMPs, thus keeping the balance between self-renewal and differentiation (Stzepourginski et al. 2017). Lastly, based on their anatomic location and properties of each organ, MCs carry out specific functions (Sanders, Ward, and Koh 2014) (Figure 5).

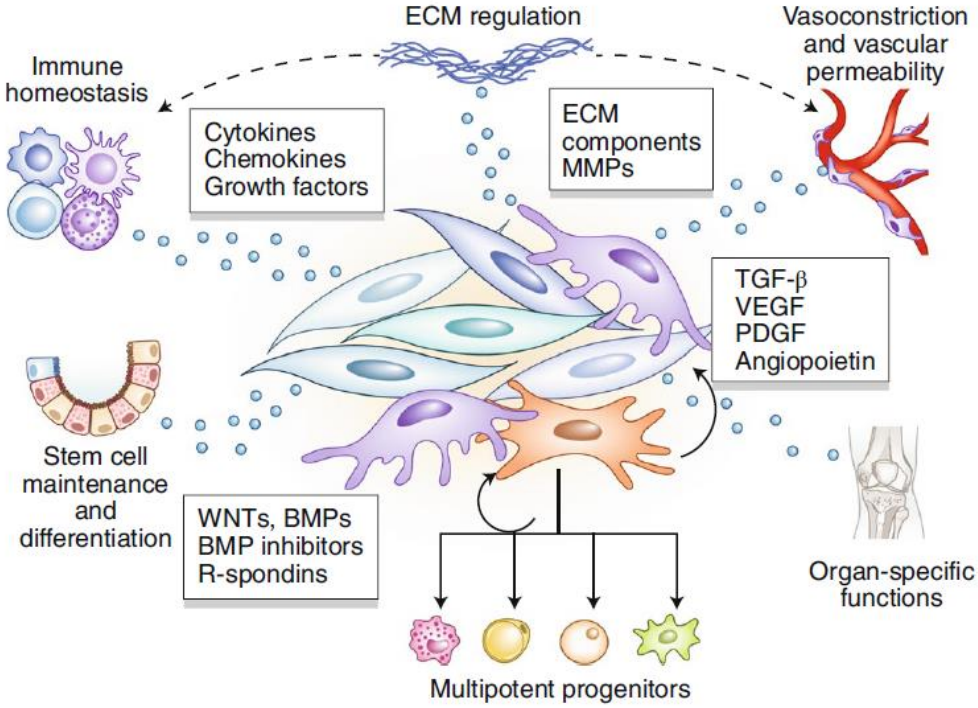

Figure 5: Homeostatic roles of mesenchymal cells (Adapted from Koliaraki et al. 2020)

Different states of fibroblast activation

Fibroblasts constitute an integral part of the microenvironment (tumor or normal). They have the ability to pass through different stages of activation and possibly de-activation. In steady state, fibroblasts are quiescent and are identified by their spindle shape, residing in the ECM. When tissue damage occurs, fibroblasts are stimulated and temporarily activated in order to aid in the process of repair and regeneration. As a result of this activation, normal activated fibroblasts (NAFs) undergo changes such as the expression of α-smooth muscle actin (αSMA) and vimentin and a shift towards a stellate shape. They also increase their production of extracellular matrix (ECM), undergo cytoskeletal rearrangements, and gain contractile properties. These new synthetic functions help to increase the fibroblasts' activation, recruitment, and proliferation through the amplification of their secretory and migratory functions. Once the repair process is complete, the activated fibroblasts' reversibility may be mediated through cellular reprogramming or apoptosis. The evolving microenvironment milieu of fibrosis and tumors is made more complex by the functionally diverse population of activated fibroblasts, known as fibrosis-associated fibroblasts (FAFs) and cancer-associated fibroblasts (CAFs). These cells not only gain enhanced proliferative properties but also exhibit specialized ECM remodeling abilities, robust autocrine activation, and dynamic immunomodulatory signaling functions. Development of cancer lesions and persisting injurious stimuli are key factors that lead to the continued activation of such fibroblasts. Epigenetic regulation may also limit their regression from this activated state (Kalluri 2016) (Figure 6).

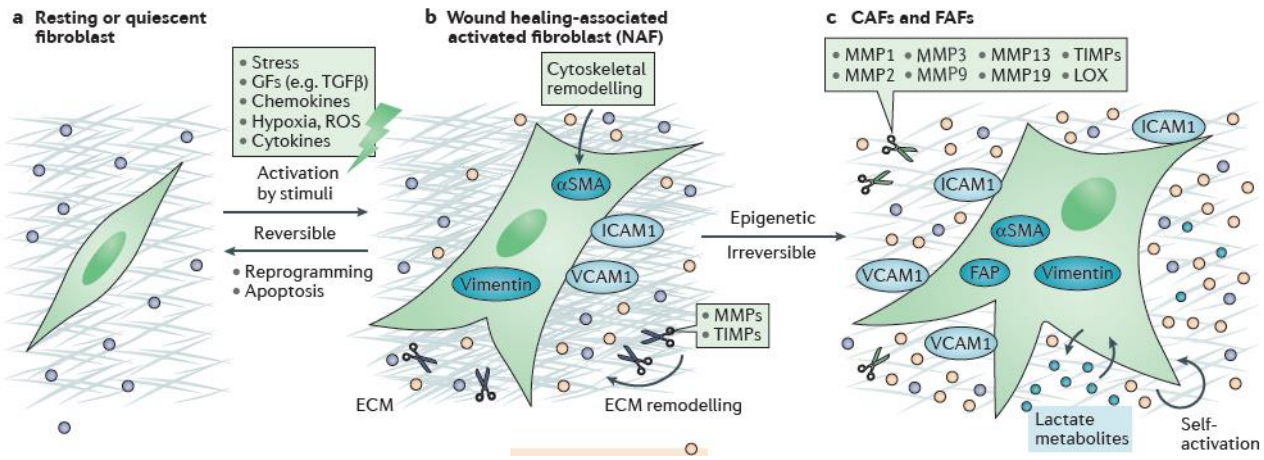

Figure 6: Multi-step activation of fibroblasts (Adapted from Kalluri 2016)

Mesenchymal cells of the intestinal lamina propria

In addition to the epithelium, the underlying mesenchyme also plays an important role in maintaining intestinal stem cells. Myofibroblasts, fibroblasts, and smooth muscle cells provide not only structural support but also soluble signals such as WNT, BMPs, BMP antagonists, and cytokines that regulate stem cell behavior. In fact, the epithelium is dependent on the presence of mesenchymal cells (Gehart and Clevers 2019). More specifically, fibroblasts have the ability to produce and remodel the ECM in order to support the structure and integrity of the intestine, while at the same time they preserve the balance between the stem cell pool and differentiated epithelium, control the immune responses and aid the endothelial functions (Koliaraki et al. 2020; Powell et al. 2011).

Myofibroblasts

Connected by both gap junctions and specialized adherens junctions containing OB-cadherin (Hinz et al. 2004), the cells attach themselves to adjacent myofibroblasts. Most crucially, these cells connect to the surrounding matrix through a fibronexus structure, rather than basal lamina. An adhesion structure, the fibronexus, binds the myofibroblast to the matrix through extracellular fibronectin filaments that extend horizontally into the cytoplasm of the myofibroblast. This forms a flat structure that serves as a plaque where fibrils can enter the cell membrane. Their matrix adhesions and cell-to-cell connections give them considerable contractile power, potentially serving a teleological purpose by reducing wound size (C. Li and Kuemmerle 2014).

At the base of crypts reside intestinal epithelial stem cells (ISCs) which are cared for, organized, and preserved by pericryptal myofibroblasts. Pericryptal myofibroblasts hold the highest standing compared to any other intestinal MSC as they boast the most "stemness" attributes. Conversely, intercryptal MSCs are a byproduct of pericryptal myofibroblasts and are mainly involved in aiding in structural functions (Valtieri and Sorrentino 2008; Signore et al. 2012).

Fibroblasts

In regard to sub-epithelial fibroblasts, their appearance can take one of two forms: spindle-shaped cells with small cytoplasmic extensions or cells with an amplified cytoplasm full of rough endoplasmic reticulum and Golgi apparatus (Toyoda et al. 2008). Although there are also intermediate appearances that are observed. As well as a large amount of rough endoplasmic reticulum, these fibroblasts hold collagen secretory granules, attach themselves to the extracellular matrix through focal adhesions, and lack smooth

muscle myofilaments and external lamina (Eyden, Curry, and Wang 2011). All of these qualities make them strong candidates for ECM component secretion rather than contractions. These cells are located in dense concentrations towards the top of the crypts and closely adjacent to epithelial cells' basolateral surface (Kurahashi et al. 2013). Although it was believed that fibroblasts were a unique population, recent single-cell RNA sequencing studies revealed an unanticipated heterogeneity of intestinal fibroblasts in homeostasis (Brügger and Basler 2023; Chalkidi, Paraskeva, and Koliaraki 2022).

Smooth Muscle Cells

Within the muscularis propria, there lies a group of cells known as intestinal smooth muscle cells. This area plays a significant role in the development of fibrosis, a process resulting in a thickening marked by cellular hyperplasia, collagen deposition, and hypertrophy. It is important to note that the muscularis propria is, in fact, the most extensive mesenchymal cell compartment in the intestine. While the smooth muscle cells in the muscularis mucosa are also present, they are lost in the affected regions of the intestine where there has been development of strictures. Smooth muscle cells, which are recognized by their positive markers of α-SMA, desmin, and collagen I, contribute significantly to fibrosis and stricture formation (Pinchuk et al. 2010).

Roles of the mesenchyme in the intestine

Regulation of epithelial cell fate determination

Recent studies highlight the importance of fibroblasts in the regulation of epithelial responses during tissue damage and repair. Based on the anatomical location and expression profile of each subset, fibroblasts can trigger different mechanisms. A diverse pool of molecules, including growth factors, cytokines and ECM remodeling enzymes is responsible for the downstream effects. For example, Tpl2 induces the activation of stromal-derived Ptgs2 and downstream PGE2 in response to innate stimuli. This phenomenon leads to improved intestinal healing and compensatory proliferation after TNBS- or DSS-induced epithelial injuries (Roulis et al. 2014). Another example is after DSS- or irradiation-induced damage, the restoration of epithelial cells was found to depend on the presence of membrane-bound MMP17, a protein that cleaves periostin and activates YAP in epithelial cells and is expressed by Grem1+ mesenchymal cells (Martín-Alonso et al. 2021). In addition, closure of gaps in an ex vivo intestinal wound healing model can be driven by the organized and directed movement of epithelial cells, which is orchestrated by direct physical interaction. This interaction includes the generation of aSMA contractile stress fibers by fibroblasts and deposition of collagen paths (Fernández-Majada et al. 2021).

Maintaining the self-renewal capacity of epithelial stem cells within the gastrointestinal (GI) tract is the primary function of the Wnt pathway. Mutual interactions between the stem cell and its local microenvironment, known as the stem cell niche, are responsible for conferring stem cell attributes. To achieve this, the niche is instrumental in regulating the Wnt signaling threshold in the stem cell to maintain a physiological range. The activity of Wnt is the highest in the intestinal stem cell niche at the bottom of the crypts, where it acts as the major regulator of ISC self-renewal and differentiation, and decreases in a gradient along the crypt–villus axis (Gehart and Clevers 2019; Gregorieff et al. 2005). The Wnt pathway also facilitates multiple feedback loops that balance cell proliferation and differentiation, thus maintaining equilibrium. The best studied “canonical” pathway depends on β-catenin as its key effector (Krausova and Korinek 2014). Wnt molecules bind to a receptor complex composed of a seven-transmembrane receptor of the Frizzled family and co-receptor low density lipoprotein receptor-related protein (LRP) (Schweizer and Varmus 2003). Then the downstream pathway is activated to the final target being β-catenin. There is evidence that supports the importance of Wnt signaling in the maintenance of

stem cell niche. For example there are studies reporting the collapse of the intestinal architecture occurs when the Wnt cascade is counteracted through ectopic expression of the secreted Wnt antagonist Dkk1 (Pinto et al. 2003; Kuhnert et al. 2004) or genetic disruption of β-catenin (Ireland et al. 2004; Fevr et al. 2007). Moreover, in a recent study the impact of CD44 on the regulation of Wnt signaling during homeostasis of the intestinal epithelium and regeneration upon injury was investigated. It was showed that a positive feedback function of CD44 boosts the Wnt signaling pathway during high Wnt signaling demand, as required for intestinal regeneration (Walter et al. 2022).

Another well studied mechanism is the R-spondin (RSPO) path. R-spondins play a pivotal role in the intricate regulation of the intestine, making them essential factors in maintaining intestinal homeostasis and overall health. These proteins are a family of secreted molecules that act as potent activators of the Wnt signaling pathway, a critical signaling cascade involved in various cellular processes, including cell proliferation, differentiation, and tissue regeneration. RSPOs form aternary complex with their LGR4/5 receptor and transmembrane E3 ubiquitin ligase proteins ZNRF3 and RNF43, thereby inhibiting turnover of the Wnt receptors. In the context of the intestines, R-spondins exert their influence on the intestinal stem cells, which are responsible for the continuous renewal of the intestinal epithelium. This robust stimulation of stem cell proliferation ensures the constant replenishment of the epithelial lining, enabling the intestines to efficiently absorb nutrients, provide a protective barrier against harmful pathogens, and maintain a healthy balance of gut microbiota (Peng et al. 2013; Wang et al. 2013; Chen et al. 2013; H.-X. Hao et al. 2012; K.-A. Kim et al. 2006). *Rspo3* is the most highly expressed RSPO in the intestine (Kabiri et al. 2014). With the use of a neutralizing antibody, it was observed that blocking RSPO3 resulted in a reduction of Lgr5 expression, providing evidence that Rspo3 plays a supporting role in this process (Storm et al. 2016). Crypt regeneration was noticeably delayed upon exposure to stress when RSPO2 and RSPO3 were combinedly neutralized, resulting in a greater inhibition of Lgr5 expression (Storm et al. 2016). A recent study revealed that subepithelial myofibroblasts marked by PdgfRα expression are an essential source of Wnts as well as a critical source of RSPO3 (Greicius et al. 2018).

In contrary to Wnt signaling pathway, BMPs, members of the TGFβ superfamily of ligands, counteract the proliferative signals of the intestinal stem cell niche and promote cell differentiation. Binding of BMPs to their type II receptors leads to subsequent activation and phosphorylation of type I BMP receptors. In turn, these receptors phosphorylate SMADs and translocate to the nucleus, where they regulate gene expression (Massagué 2012). In the intestine, BMP2 and BMP4 are the main ligands for BMP receptors. Both ligands are secreted by intercrypt and intervillus mesenchymal cells (Hardwick et al. 2004; Haramis et al. 2004). Ectopic overexpression of the BMP inhibitors Gremlin 1 or Noggin in the intestinal epithelium in mice causes excessive ectopic crypt formation and growth of polyps(Haramis et al. 2004; Davis et al. 2015).

Active Notch signalling requires direct membrane contact between two cells, one expressing Notch ligands and the other Notch receptors (Sancho, Cremona, and Behrens 2015). Upon receptor activation, the Notch intracellular domain (NICD) is proteolytically released by γ- secretase and translocates to the nucleus, where it binds the transcription factor CSL. The main downstream effect of Notch signalling is expression of the basic loop helix transcription factor HES1 (Sancho, Cremona, and Behrens 2015). Maintaining the stem cells at the bottom of the crypt and regulating the ratio of secretory to absorptive progeny occurs due to Notch signalling in the intestine. This signalling pathway blocks stem cell differentiation into the secretory lineage. At the base of the crypt and in transit amplifying cells nearest to the stem cell region, nuclear NICD staining reveals elevated Notch signaling activity in CBC cells (Tian et al. 2015). In mice, if NEUROG3+ enteroendocrine precursor cells have Notch signalling activated, the resulting cells mainly consist of goblet cells and enterocytes with minimal enteroendocrine cells (H. J. Li et al. 2012).

Regulation of immune responses

It is known that upon damage or inflammation, fibroblast activation is triggered, resulting in the expression of various pro-inflammatory genes and downstream recruitment of immune cells. The most known activators of intestinal fibroblasts are IL-1β and TNF (Roulis et al. 2014; Friedrich et al. 2021). Moreover, in the activation of fibroblasts, NFkB and MAPK pathways play a vital role as downstream mediators. During DSS colitis, the activation of PDGFRa^{hi} fibroblasts is greatly dependent on NFkB signaling, with Col6a1-Cre-specific IKK2 deletion leading to diminished colitis. A decrease in the production of inflammatory mediators, less infiltration of inflammatory cells, and the activation of epithelial-specific STAT3 were the primary reasons for this decrease (Koliaraki, Pasparakis, and Kollias 2015). Although intestinal fibroblasts possess a pro-inflammatory role, they also contribute to the resolution of inflammation through different mechanisms. Specifically, they can secrete anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β). These cytokines can suppress the activity of pro-inflammatory immune cells and promote the resolution of inflammation (Steen et al. 2020). In addition, they synthesize and deposit ECM components, including collagen and fibronectin, providing structural support and acting as a barrier that helps to prevent the infiltration of immune cells into inflamed tissues (Speca et al. 2012). Moreover, ECM produced by iMCs acts as a scaffold that leucocytes adhere to and crawl along after extravasating from the blood (Vaday and Lider 2000). iMCs can also directly contribute to leucocyte migration by producing chemokines, and they express numerous chemokine genes in the steady-state intestine of both humans and mice. These include the genes encoding CXCL13, the B-cell and lymphoid tissue inducer cell chemoattractant; the myeloid cell chemoattractants CCL2, CCL8, CCL11 and CCL13 and the pleiotropic primordial chemokines CXCL12 and CXCL14 (Kinchen et al. 2018; Thomson et al. 2018; Karpus et al. 2019). Furthermore, evidence has revealed that cultured human and murine myofibroblasts produce the inflammatory cytokine IL-6, upregulate the neutrophil chemoattractants IL-8/CXCL8 or CXCL1, and upregulate cyclo-oxygenase 2 (COX2) to produce prostaglandin E_2 (PGE_2) in response to lipopolysaccharide (LPS) (Zhang et al. 2005; Walton, Holt, and Sartor 2009; Rogler et al. 2001). Mucosal integrity is maintained, bacterial dissemination is limited, and intestinal inflammation is reduced through TLR2 expression by non-haematopoietic cells when clearing C. rodentium. In fact, rather than amplifying local inflammatory responses, this is required (Gibson et al. 2010).

Mucosal inflammation is pivotal for host defence and pathogen clearance, but it comes at a potential cost, because the production of cytokines, matrix metalloproteinases (MMPs), reactive oxygen species (ROS) and nitrogen intermediates can lead to pathological ECM remodelling, epithelial cell death and ulceration. Some of the characteristic cytokines produced by iMC during inflammation, such as IL-11 and IL-33, can have pro-repair and pro-inflammatory functions depending on the context. In protecting against intestinal inflammation caused by C. rodentium, IL-11 is produced by α-SMA+ iMC and subepithelial myofibroblasts within the muscularis mucosa, promoting wound repair and preventing epithelial damage (Gibson et al. 2010). Interleukin-33 is expressed by numerous stomal cell populations in the steady-state intestine and plays an important role in maintaining epithelial barrier integrity in the intestine (Mahapatro et al. 2016). IL-33 is upregulated by peri-cryptal iMC during *Salmonella typhimurium* infection in mice and it helps protective immunity by driving expansion of secretory epithelial cells and promoting epithelial antimicrobial defence. However, IL-33 is also upregulated in the inflamed mucosa of patients with UC, predominantly by the α-SMA$^+$ iMCs that accumulate within lesions (Sponheim et al. 2010; Kobori et al. 2010).

Support of blood vessel structure and function

Mural cells and endothelial cells make up all blood vessels. The inner wall of the vessel is formed by endothelial cells, while the mural cells coat and associate with the endothelial cell tube. Mural cells are further categorized as vascular smooth muscle cells or pericytes based on factors such as their location, morphology, expression of specific markers, and density. Multiple concentric layers of vascular smooth muscle cells surround arteries and veins, whereas pericytes are associated with the smallest blood vessels including arterioles, capillaries, and venules, sharing their basal membrane with the endothelium (Gaengel et al. 2009). The ligand/receptor pair platelet-derived growth factor (PDGF)B/PDGF receptor-beta (PDGFRβ) are factors with relatively clearly defined roles during pericyte recruitment (Andrae, Gallini, and Betsholtz 2008). PDGFB is secreted as a homodimer from the endothelium of angiogenic sprouts where it serves as an attractant for comigrating pericytes, which in turn express PDGFRβ (Lindahl et al. 1997). In addition, PDGFB stimulates proliferation of vascular smooth muscle cells and induces mural cell fate in undifferentiated mesenchymal cells (Bjarnegård et al. 2004; Abramsson, Lindblom, and Betsholtz 2003). The signaling loop of the PDGFB/PDGFRβ axis has been identified as paracrine communication between endothelium and mural cells, while the angiopoietin-Tie receptor axis functions primarily in the opposite direction, transmitting signals from mural cells to endothelial cells. All angiopoietins (Angs) are ligands for the Tie2 receptor (Gaengel et al. 2009). These two main paths are important for regulation of vasoconstriction and normal vascular permeability.

ECM remodeling

The ECM is constantly renewed and remodeled by coordinated action among ECM-producing cells, degrading enzymes, and their specific inhibitors. During this process, several growth factors are released in the ECM, and they, in turn, modulate the deposition of new ECM. Fibroblasts are the main source of most of the proteins composing ECM, such as collagens, fibronectin and hyaluronan. Matrix metalloproteinases (MMPs), Lysyl oxidases (LOXs) and tissue-inhibitor of metalloproteinases (TIMPs) are the major groups of enzymes acting to remodel the ECM and enable cellular migration and release of the bound growth factors in the ECM reservoir (Bonnans, Chou, and Werb 2014). Upon tissue injury and regeneration, mesenchymal cells are activated resulting in differential expression of cytokines and chemokines to attract immune cells and remodeling enzymes. In a DSS injury model, expression levels of MMP-2 and -9 were increased 2 days following cessation of DSS exposure (Breynaert et al. 2016). Expression of TIMP-1 and -2 was also found upregulated at the restitution phase, which may act to inhibit the degrading action of the MMPs during acute wound healing response (Strup-Perrot et al. 2006). Interestingly, DSS-injured TIMP-1 knockout animals recovered significantly faster than wildtype despite the initial increase in MMP activity and inflammation, suggesting that the balance between MMPs and their inhibitors is complex and critical for intestinal regeneration (Breynaert et al. 2016). The ECM can act as a reservoir for growth factors and cytokines. These proteins may be released or activated upon MMP-mediated proteolysis of the ECM following injury (Hynes 2009). Remodeling of the ECM enhances cell migration, which can result in increased localized concentrations of growth factors as a consequence of mesenchymal cell infiltration.

In IBD, ECM goes through various compositional changes. Mesenchymal cells of the lamina propria are altered by the fibrogenic phenotype with increased abundance of myofibroblasts, leading to enhanced ability to trigger ECM synthesis (Gelbmann et al. 1999). Excessive secretion of TGF-β has been linked to increased deposition of fibronectin, which triggers the activation, migration, and proliferation of fibroblasts into myofibroblasts. Interestingly, studies have also demonstrated that fibronectin interacts with TNF-α to enhance monocyte-derived MMP-9 expression and chemotactic response (Vaday et al.

2000). Inhibition of proteolytic enzymes presents valuable therapeutic potential when considering the significant increase of ECM fragmentation by MMPs in inflamed IBD tissue (O'Sullivan, Gilmer, and Medina 2015). Studies indicate that inflamed Crohn's tissue has downregulated inhibitors of MMPs, including TIMP-3. Likewise, depletion of TIMP-3 in mice promotes development of colitis (Monteleone et al. 2012).

Decellularization of the intestine

The need of evolving from 2D culture to an *ex vivo* context was becoming a necessity in the previous years. This is when organotypic cultures, multicellular fragments that contain the major cell types of a particular organ and resembles it's *in vivo* organization, emerged (de Souza 2017). Matrigel is the most common tool for growing gastrointestinal (GI) organoids in vitro. However, due to its nature, it has some drawbacks. First, it is a raw material that comes from a specific type of cancer and causes large batch-to-batch variation in organoids. Moreover, its composition is different from that of normal tissues and, thus, may not provide the appropriate microenvironment for the organoids to grow. Alternatives to Matrigel like synthetic poly (ethylene glycol) (PEG) or natural hydrogels have been tested but they did not fully reconstitute the 3D microenvironment. Recently, a new material for GI organoids culture has been developed and this is GI-derived extracellular matrix hydrogels. Using decellularization techniques, Kim et al. managed to develop a platform that mimics the native GI microenvironment. It overcomes all the disadvantages of Matrigel and is more cost-effective (S. Kim et al. 2022).

Recent studies have demonstrated the applicability of the decellularization method and the remarkable possibilities it offers in the progression of regenerative medicine. More particularly, (Giuffrida et al. 2019) showed that the decellularized human intestine can serve as an effective 3D scaffold for studying intestinal fibrosis. The decellularized intestine preserved the structure and composition of the original tissue, allowing the cultivation of intestinal fibroblasts and the study of underlying mechanisms of fibrosis (Giuffrida et al. 2019). Moreover, (Meran et al. 2020)described a novel approach for generating transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure. This study demonstrated the feasibility of using organoids derived from the intestinal crypts of pediatric patients with short bowel syndrome to create functional, transplantable jejunal mucosal grafts. Grafts exhibited normal epithelial differentiation and maturation, and when transplanted into recipient mice, the grafts integrated into host tissues and formed functional intestinal epithelium (Meran et al. 2020).

MATERIALS AND METHODS

Mice and ethical statements

Mice were maintained on a C57BL/6 genetic background, and experimental groups contained littermates that were caged together according to gender. Experiments were performed in the conventional unit of the animal facilities in Biomedical Sciences Research Center (BSRC) "Alexander Fleming" under specific pathogen–free conditions, in accordance with the guidance of the Institutional Animal Care and Use Committee of BSRC Alexander Fleming".

Induction of DSS acute colitis

In brief, dextran sodium sulfate (DSS) acute colitis was induced in 8-week or older mice with 2.5% DSS (MP Biomedicals) added in the drinking water for 5 days, followed by 2 or 9 days of normal

water for acute colitis (Wirtz et al. 2007) and regeneration phase accordingly. Mice were weighted on day 1 and every day since the removal of DSS.

Isolation of intestinal mesenchymal cells (IMC) from mice

For isolation of IMCs, colons from 8-week-old mice were removed. Briefly, animals were euthanized and colons were cut right before the cecum, flushed with ice cold 1X Phosphate Buffered Saline (PBS) +Penicillin/Streptomycin/Antimycotic (P/S/A) and cleared from fat and mesentery. They were then cut longitudinally, placed in ice cold 1X PBS (+P/S/A) and washed 3 times. Colons were then incubated in pre-warmed Hanks' Balanced Salt Solution (HBSS) containing 5 mM EDTA (Fisher) and 1 mM Dithiothreitol (DTT) (Applichem) for 25 min at 37°C to remove the epithelial layer. Colons were then washed approximately 5 times with 1X PBS (+P/S/A) and cut in 0.5–1 cm pieces. This was followed by incubation with 300 U/ml Collagenase XI (Sigma-Aldrich) and 0.1 mg/ml Dispase (Roche) in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) for 60 min at 37°C. Every 10 minutes the falcons were shaken vigorously. Supernatants were then filtered through a 70µm cell strainer, centrifuged (4 ◦C, 300g, 6minutes) and cell pellets were resuspended in DMEM supplemented with 10% Fetal bovine serum **(**FBS) (Biochrom), 1% nonessential amino acids (Sigma-Aldrich), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1 µg/ml amphotericin B (Sigma-Aldrich) and plated in cell culture flasks. At passages 3–4, cells were used for downstream experiments.

Decellularization

Intestinal tissues were obtained from C57BL/6 mice. Fat tissues, large blood vessels and mesentery were removed prior to decellularization. The small and large intestine were resected and the large intestine was cut longitudinally. The intestine was thoroughly shaken with MilliQ water to wash away the remaining stools. Then, it was incubated in MQ water overnight at 4^{o}C, followed by flushing with MQ water. The intestine was then cut into smaller pieces (~1cm), which were incubated with 1% Sodium Deoxycholate (43035, BDH Chemicals Ltd Poole England) for 3h at RT on a shaker, washed with MQ water for 15 min at RT on a shaker and subsequently incubated with 1M NaCl and DNase1 (1U/10µl) for 2h at RT on a shaker. The pieces of iECM were then washed with PBS 1x for 15min at RT on a shaker and stored in storage buffer or PBS 1x with P/S/A at 4^{o}C (short-term usage) (Iqbal et al. 2021).

- Storage buffer: P/S (1%) and sodium azide (0,3µM) in ddH_2O.

DNA extraction from tissue

The matrix was digested by incubating in 400 µl of Digestion buffer and 6µl of proteinase K (RP100B, BLIRT) with a final concentration of 0.15mg/ml overnight at 56^{o}C. Then, phenol was added to the samples. The samples were shaken well and centrifuged for 10min at 13.000rpm. The upper (transparent) phase of the samples was transferred to a new tube and mixed with isopropanol at 1:1 ratio, followed by centrifugation for 10min at 13.000rpm. The supernatant was removed and 70% ethanol was used to wash the pellets followed be a centrifugation for 5min at 13.000rpm. The pellet was left to dry for 20min and 80-200µl ddH_2O was used to resuspend the samples. Concentration of the DNA of the samples was then measured using a NanoDrop spectrophotometer (Thermo Scientific).

- Digestion buffer: Tris 1M pH 8.0, EDTA 0.5M, NaCl 5M, SDS 10%

RNA extraction from tissue

For RNA extraction from tissue, the RNeasy Mini Kit by Qiagen was used. In brief, samples were first lysed and then homogenized. Ethanol was added to the lysate to provide ideal binding conditions.

The lysate was then loaded onto the RNeasy silica membrane and RNA was bound to the silica membrane, and all contaminants were efficiently washed away. The residual amounts of DNA remaining were removed using on-column DNase treatment. Pure, concentrated RNA was eluted in water.

Immunostaining

The pieces of the decellularized tissue were placed in a 24-well plate. Blocking buffer was added to the samples and left overnight on a rocking table at RT. Then, the primary antibody was added to the samples for 20-24h on a rocking table, followed by 5 washes with the washing solution, for 1 h each. Then, the secondary antibody was added to the samples, protected from the light and left for incubation overnight on a rocking table at RT, followed by 3 washes as previously described. Lastly, the samples were left in storage buffer for confocal imaging using the Leica TCS SP8 Confocal microscope (Mayorca-Guiliani et al. 2019).

- Blocking buffer: 3% Bovine serum albumin **(**BSA) in 1x PBS
- Primary antibody solution: rabbit anti-mouse Collagen IV antibody (Abcam, ab6586, 1:500 in blocking buffer)
- Secondary antibody solution: anti-rabbit Alexa 488-conjugated antibody (A11008, Invitrogen, 1:500 in blocking buffer), DAPI (d1306, Invitrogen, 1:1000)
- Washing solution: 0,05% Tween 20 in 1x PBS
- Storage buffer: P/S (1%) and sodium azide (0,3μM) in ddH_2O.

Digestion of the tissue for cell counting

Pieces of the intestine (both normal and decellularized) were digested with Collagenase XI (C7657, Sigma-Aldirch) and Dispase II (04942078001, Roche Diagnostics) (5ml/piece) at 37^{o}C. The decellularized tissue was digested for 15min, while the normal for 60min. The digested samples were each diluted with 20ml of DMEM, filtered using a 70μm cell strainer and cells were counted on the coulter cytometer (BECKMAN COULTER Life Sciences).

- Digestion solution: 300 U/ml collagenase XI and 0,08 U/ml dispase II dissolved in DMEM

Gelation of decellularized intestine

Decellularized pieces passed through a dehydration process by centrifugation in the SpeedVac SPD1030. Then, the powder was weighted in order to calculate the appropriate volume of Pepsin/HCl to be used. Pepsin (Sigma-Aldrich) was diluted in 0.01M HCl to final concentration of 1mg/ml and stored in aliquots. This mixture was then subjected to digestion by mortar and incubation for 72h in 70rpm, RT. The incubation was followed by a centrifugation at 13.000rpm for 10min, RT. Then the supernatant was collected and the pellet was rejected. After that, supernatant was sterilized by UV in the culture hood. Final step was to regulate the osmomolarity/salinity with 10X PBS to be compatible for cell culture and neutralization of pH to 7.4 with 0.01M NaOH (Giobbe et al. 2019).

Aim of the study

The aims of this thesis were i) to standardize the protocol for the decellularization of the mouse intestine and the characterization of its proteome, ii) to characterize the changes in the ECM composition of the colon during acute DSS colitis and subsequent intestinal regeneration, and to iii) establish a

protocol for the generation of a hydrogel matrix from mouse decellularized intestine that could be used in the culture of IMCs.

RESULTS

Standardization of the decellularization protocol

Normal mouse colon was used for the standardization of the decellularization protocol, according to the protocol published by Iqbal et al (2021). To verify the efficiency of the method, the DNA content of 1 piece of normal tissue and 2 pieces of decellularized tissue was measured, showing a significant reduction in the DNA content of the decellularized tissue (Figure 7).

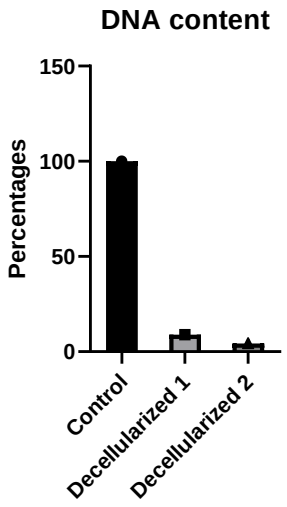

Figure 7: Percentage of DNA content compared to control.

To further validate these results, 2 pieces of normal tissue and 2 pieces of decellularized tissue were digested with Collagenase XI and Dispase II and cells were counted. The decellularized tissues contained 10-100-fold less cells than the normal tissues (Figure 8).

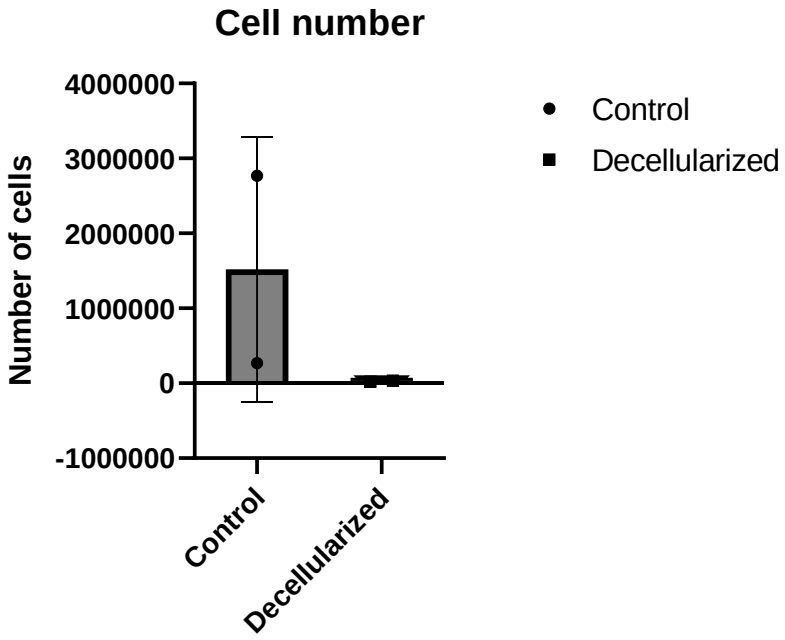

Figure 8: Cell numbers in the decellularized tissue compared to control.

Finally, staining of the decellularized tissues with an antibody against Collagen IV showed the matrix network, while DAPI staining verified the absence of cells (Figure 9 – diffused non-specific staining).

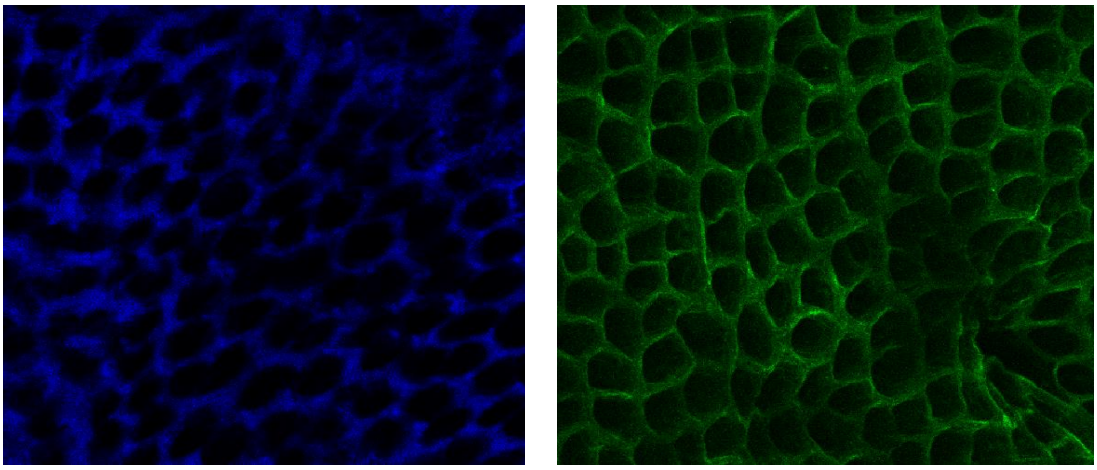

Figure 9: Representative images of the decellularized colon stained with an antibody against Collagen IV (green). DAPI was used to stain the nuclei blue (only background is visible) (20x magnification).

Proteomic analysis of the decellularized colon

After verifying the efficiency of the decellularization protocol, one piece (1cm) of the decellularized tissue was sent for LC-MS/MS analysis to analyze its proteomic composition. The analysis

was performed in the Proteomics Facility of BSRC "Alexander Fleming". The results from the analysis were compared with a matrisomal signature from Naba et al. where they have characterized ECM proteins from human and mouse tissues (Naba et al. 2012). According to Naba et al, ECM proteins are divided into two main categories: 1) Core Matrisome and 2) Matrisome-associated proteins, and each one is split into 3 sub-categories.

We were able to detect 157 matrisomal proteins in our decellularized tissue, 97 proteins were characterized as core matrisome proteins from normal colon and lung based on Naba et al. Similarly, 60 proteins were designated as matrisome-associated. 66% of the core-matrisome proteins from our sample are ECM glycoproteins while the other 13% are proteoglycans and 21% collagens. 42% of the matrisome-associated proteins from our sample are ECM regulators, 26% are secreted factors and 32% are ECM affiliated proteins (Figure 10).

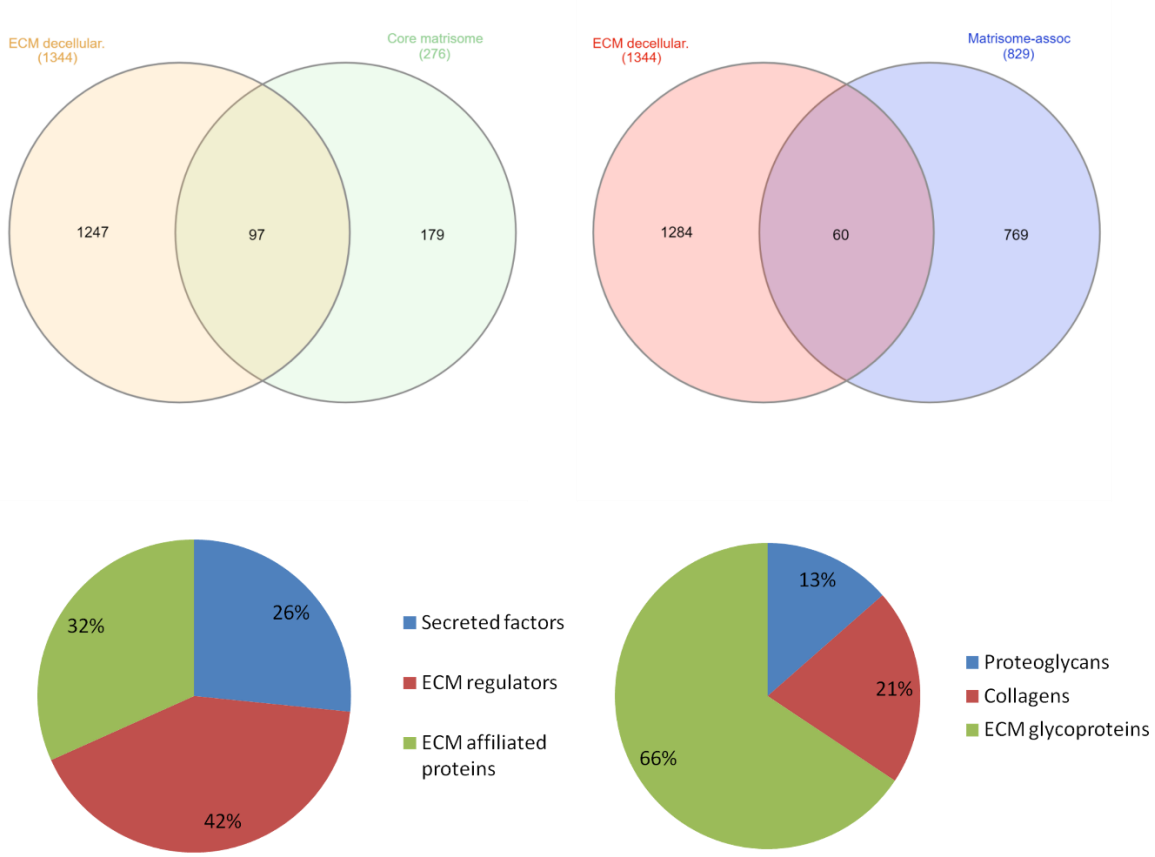

Figure 10: Venn diagrams showing the comparison between the proteins from our decellularized intestinal sample and the core matrisome proteins (Upper left) and to matrisome-associated proteins (Upper right) from Naba et al (2012). Also, pie charts are shown, where the 60 and 97 common ECM decellularized proteins from our sample were compared to the matrisome-associated (down left) and to the core matrisome (down right) subcategories from mouse colon from Naba et al (2012).

ECM changes during intestinal damage and repair

To identify differences in ECM during intestinal inflammation and regeneration, we harvested colons from mice at different timepoints of the DSS protocol and performed decellularization, as previously described. We collected 5 samples per condition (control – Day 0, acute inflammation – Day 7,

regeneration – Day 14). Body weight (Figure 11) and colon length (Figure 12) were measured to verify colitis stage. The DNA content in each decellularized sample was measured to validate the efficiency of the protocol, as previously described (Figure 13).

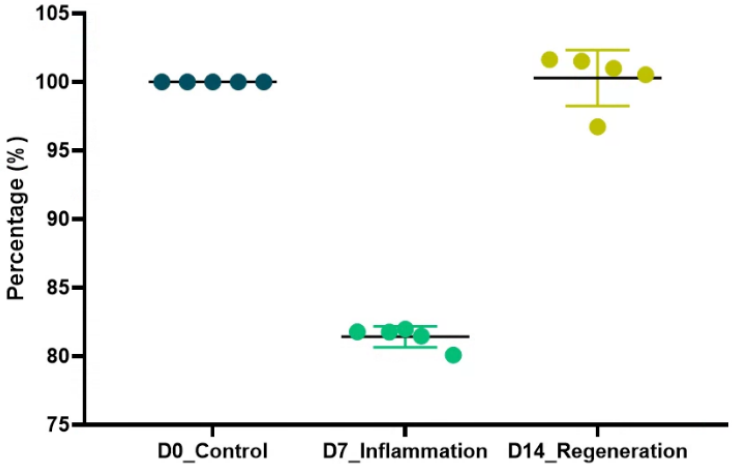

Figure 11: Body weight loss in mice after DSS treatment.

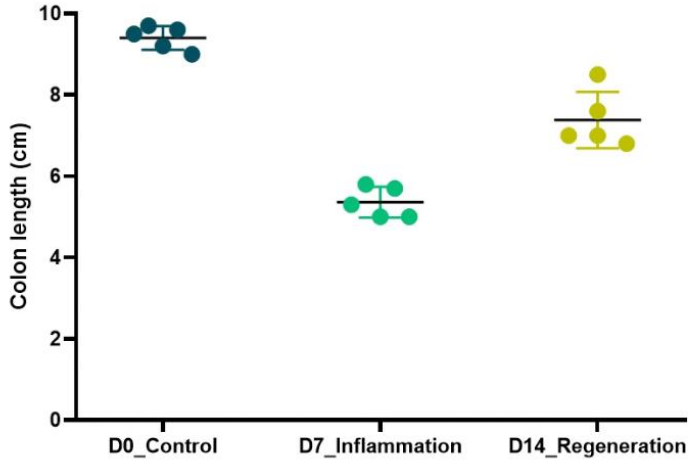

Figure 12: Colon length in the 3 different timepoints.

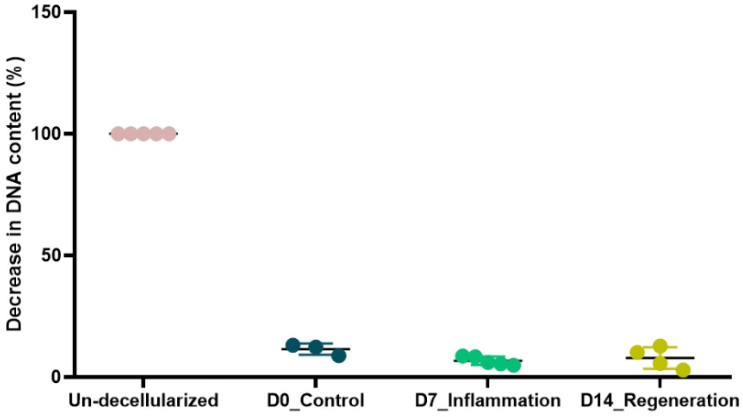

Figure 13: DNA content from colons after decellularization.

After validation of efficient decellularization, samples were used to perform proteomics analysis, using HPLC-MS/MS. In the following heatmap are presented 3 timepoints of the DSS protocol: D0, D7 and D14. These timepoints were chosen as they depict control, acute inflammation, and regeneration accordingly. We focused on ECM-related proteins and were able to detect significant differences between the different timepoints. For example, we found increased levels of MMPs during the acute phase and deregulation in the expression levels of different collagens and ECM components that require further investigation (Figure 14).

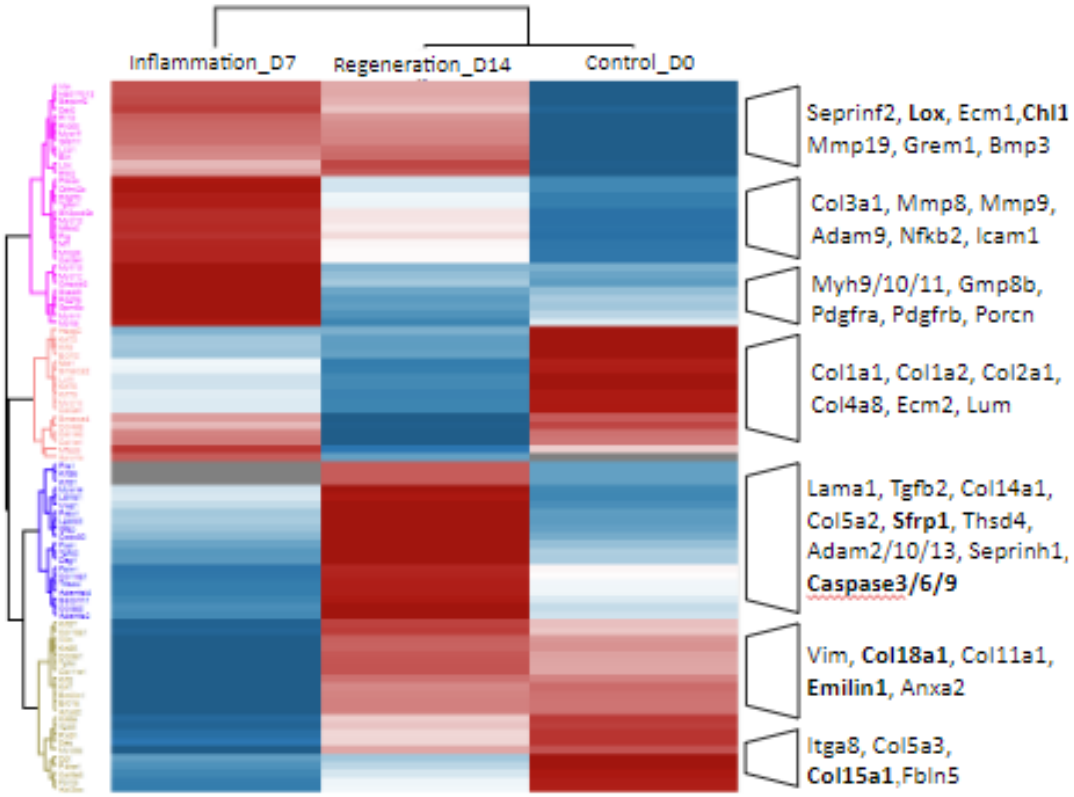

Figure 14: Heatmap showing ECM proteins, whose levels change during inflammation and regeneration.

From these data, we selected two proteins of interest, Sfrp1 and Col18a1 to determine if ECM protein level changes correlate with gene expression. To this end, we performed again DSS colitis experiments and collected tissues from 3 mice per timepoint. Monitoring of the protocol through body weight measurements is shown in Figure 15.

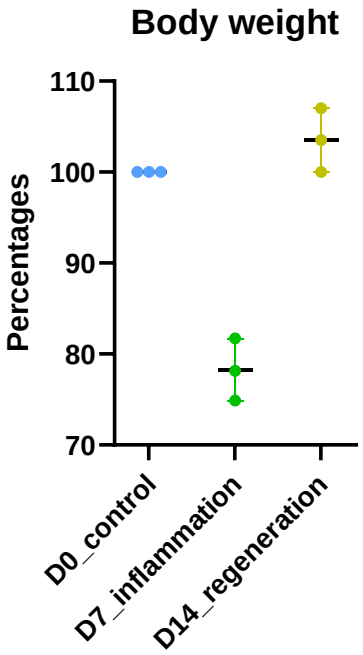

Figure 15: Body weight loss in mice after DSS treatment.

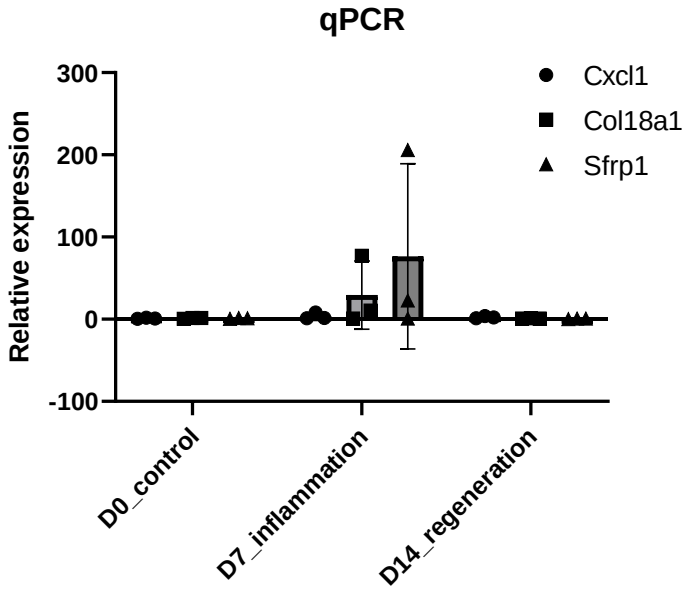

Figure 16: qRT-PCR analysis of colon tissue from different timepoints during the DSS colitis protocol, including untreated (D0, n=3), acute inflammation (D7, n=3) and regeneration (D14, n=3). Analysis was performed using 2-way ANOVA.

Analysis of gene expression by qRT-PCR revealed elevated levels of *Col18a1* and *Sfrp1* in the acute inflammation D7 phase in comparison with untreated mice, and a subsequent downregulation at the regeneration phase (D14) with no statistical significance. (Figure 16).

ECM-derived hydrogels for IMC cultures

Finally, to establish whether mouse decellularized intestinal tissue can be used as a matrix for IMC culture, we performed gelation experiments to generate hydrogels. We specifically used decellularized small intestine from normal mice. The concentration of the resulting ECM hydrogel was 10mg/ml diluted in pepsin/HCl and neutralized to a final pH=7.4. The hydrogel was plated in 96-well plates and incubated for 20-30 minutes at 37^{o}C to acquire gel-like properties. IMCs isolated from normal mice were then plated on top of the hydrogel and incubated for 48 hours. After 24 hours, IMCs had expanded and formed a monolayer (Figure 17).

Figure 17: Representative microscopy image of IMCs grown on top of ECM-derived hydrogel.

DISCUSSION

Matrigel is a popular choice for growing organoids due to its effectiveness. However, it is not without limitations. Batch variation, safety concerns, and the absence of tissue-specific biochemical ECM factors are all critical issues that should be considered. Alternatives to Matrigel like synthetic poly(ethylene glycol) (PEG) or natural hydrogels have been tested but they did not fully mimic the 3D microenvironment. Recently, a new material for GI organoids culture has been developed and this is Gastrointestinal (GI)-derived extracellular matrix hydrogels. Although relevant reports are still few, especially in the mouse GI-derived hydrogel, some studies, using porcine GI tract as material, report that the decellularized GI tissue-derived ECMs have a relatively low batch-to-batch variation, are more cost effective compared to Matrigel and most importantly hydrogels made with ECM have been determined to be safe for clinical use, with low levels of endotoxin and minimal immunogenicity observed both in vitro and in vivo. This is largely due to the absence of any xenogeneic pathogens or cellular components that could trigger an immune response (S. Kim et al. 2022).

During my dissertation thesis we set out to establish a protocol for the decellularization of the colon of mice and test its efficiency. The decellularization process was successful, verified by a significant decrease in DNA concentration and number of cells. Confocal imaging revealed the absence of cells and the presence of ColIV networks delineating crypt structures. HPLC-MS/MS analysis uncovered the protein composition of the decellularized ECM, which has a lot in common with the Matrisome of normal mouse colon, reported in Naba et al (Naba et al. 2012).

Based on the proteomic analysis of the normal ECM, we further analyzed the protein composition of decellularized colon tissue from mice treated with DSS during acute inflammation (Day 7 after initiation of DSS protocol) and regeneration (Day 14 after initiation of DSS protocol). This analysis showed significant differences between the different timepoints, especially in ECM related proteins. For example, we found increased levels of MMPs during the acute phase and deregulation in the expression levels of different collagens and ECM components that require further investigation. In addition, correlation of the proteomic data with single-cell RNA sequencing data could reveal the contribution of specific cell types in inflammation- and regeneration-associated ECM composition and function. As part of this thesis, the correlation between ECM protein levels and tissue gene expression was tested using qRT-PCR. For this purpose, SFRP1 and COL18A1 were selected. SFRP1 protein levels were elevated at Day 14, while COL18A1 levels were elevated in both timepoints in comparison to untreated controls. Gene expression analysis showed that mRNA levels of both genes were increased in acute inflammation and then dropped during regeneration, although the differences were not statistically significant. This could be attributed to the small sample size, and thus more samples should be included in the analysis. The potential difference between the RNA and protein levels, especially during regeneration could be related to protein stability and/or sequestration and release by the ECM, which warrants further investigation.

Finally, we developed hydrogels from decellularized intestinal tissue and successfully managed to grow IMCs on top. However, more work is needed to establish the efficiency and safety of mouse intestine-derived hydrogels used as substrate for the growth of cells or even organoids. For example, it is necessary to identify the optimal conditions in hydrogel composition, including concentration of pepsin/HCl and ECM gel, pH, and number of cells plated on or embedded in the hydrogels.

BIBLIOGRAPHY

- Abramsson, Alexandra, Per Lindblom, and Christer Betsholtz. 2003. "Endothelial and Nonendothelial Sources of PDGF-B Regulate Pericyte Recruitment and Influence Vascular Pattern Formation in Tumors." *The Journal of Clinical Investigation* 112 (8): 1142–51. https://doi.org/10.1172/JCI18549.
- Adams, Josephine C., and Fiona M. Watt. 1993. "Regulation of Development and Differentiation by the Extracellular Matrix." *Development* 117 (4): 1183–98. https://doi.org/10.1242/dev.117.4.1183.
- Alam, Ashfaqul, and Andrew Neish. 2018. "Role of Gut Microbiota in Intestinal Wound Healing and Barrier Function." *Tissue Barriers* 6 (3): 1539595. https://doi.org/10.1080/21688370.2018.1539595.
- Andrae, Johanna, Radiosa Gallini, and Christer Betsholtz. 2008. "Role of Platelet-Derived Growth Factors in Physiology and Medicine." *Genes & Development* 22 (10): 1276–1312. https://doi.org/10.1101/gad.1653708.
- Armulik, Annika, Guillem Genové, and Christer Betsholtz. 2011. "Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises." *Developmental Cell* 21 (2): 193–215. https://doi.org/10.1016/j.devcel.2011.07.001.
- Bamias, Giorgos, Li-Guo Jia, and Fabio Cominelli. 2013. "The Tumor Necrosis Factor-like Cytokine 1A/Death Receptor 3 Cytokine System in Intestinal Inflammation." *Current Opinion in Gastroenterology* 29 (6): 597. https://doi.org/10.1097/MOG.0b013e328365d3a2.
- Barker, Nick, Johan H. van Es, Jeroen Kuipers, Pekka Kujala, Maaike van den Born, Miranda Cozijnsen, Andrea Haegebarth, et al. 2007. "Identification of Stem Cells in Small Intestine and Colon by Marker Gene Lgr5." *Nature* 449 (7165): 1003–7. https://doi.org/10.1038/nature06196.
- Bjarnegård, Mattias, Maria Enge, Jenny Norlin, Sigrun Gustafsdottir, Simon Fredriksson, Alexandra Abramsson, Minoru Takemoto, Erika Gustafsson, Reinhard Fässler, and Christer Betsholtz. 2004. "Endothelium-Specific Ablation of PDGFB Leads to Pericyte Loss and Glomerular, Cardiac and Placental Abnormalities." *Development* 131 (8): 1847–57. https://doi.org/10.1242/dev.01080.
- Bollyky, Paul L., Ben A. Falk, Rebecca P. Wu, Jane H. Buckner, Thomas N. Wight, and Gerald T. Nepom. 2009. "Intact Extracellular Matrix and the Maintenance of Immune Tolerance: High Molecular Weight Hyaluronan Promotes Persistence of Induced CD4+CD25+ Regulatory T Cells." *Journal of Leukocyte Biology* 86 (3): 567–72. https://doi.org/10.1189/jlb.0109001.
- Bonnans, Caroline, Jonathan Chou, and Zena Werb. 2014. "Remodelling the Extracellular Matrix in Development and Disease." *Nature Reviews Molecular Cell Biology* 15 (12): 786–801. https://doi.org/10.1038/nrm3904.
- Breynaert, Christine, Magali de Bruyn, Ingrid Arijs, Jonathan Cremer, Erik Martens, Leentje Van Lommel, Karel Geboes, et al. 2016. "Genetic Deletion of Tissue Inhibitor of Metalloproteinase-1/TIMP-1 Alters Inflammation and Attenuates Fibrosis in Dextran Sodium Sulphate-Induced Murine Models of Colitis." *Journal of Crohn's and Colitis* 10 (11): 1336–50. https://doi.org/10.1093/ecco-jcc/jjw101.
- Brügger, Michael David, and Konrad Basler. 2023. "The Diverse Nature of Intestinal Fibroblasts in Development, Homeostasis, and Disease." *Trends in Cell Biology* 0 (0). https://doi.org/10.1016/j.tcb.2023.03.007.
- Cai, Zhaobei, Shu Wang, and Jiannan Li. 2021. "Treatment of Inflammatory Bowel Disease: A Comprehensive Review." *Frontiers in Medicine* 8 (December): 765474. https://doi.org/10.3389/fmed.2021.765474.
- Castro Brás, Lisandra E. de, and Nikolaos G. Frangogiannis. 2020. "Extracellular Matrix-Derived Peptides in Tissue Remodeling and Fibrosis." *Matrix Biology*, Fibroblasts: The arbiters of matrix remodeling, 91–92 (September): 176–87. https://doi.org/10.1016/j.matbio.2020.04.006.
- Caswell, Patrick T., and Tobias Zech. 2018. "Actin-Based Cell Protrusion in a 3D Matrix." *Trends in Cell Biology* 28 (10): 823–34. https://doi.org/10.1016/j.tcb.2018.06.003.
- Chalkidi, Niki, Christina Paraskeva, and Vasiliki Koliaraki. 2022. "Fibroblasts in Intestinal Homeostasis, Damage, and Repair." *Frontiers in Immunology* 13. https://www.frontiersin.org/articles/10.3389/fimmu.2022.924866.
- Chen, Po-Han, Xiaoyan Chen, Zhenghong Lin, Deyu Fang, and Xiaolin He. 2013. "The Structural Basis of R-Spondin Recognition by LGR5 and RNF43." *Genes & Development* 27 (12): 1345–50. https://doi.org/10.1101/gad.219915.113.

- Cheng, H., and C. P. Leblond. 1974. “Origin, Differentiation and Renewal of the Four Main Epithelial Cell Types in the Mouse Small Intestine. V. Unitarian Theory of the Origin of the Four Epithelial Cell Types.” *The American Journal of Anatomy* 141 (4): 537–61. https://doi.org/10.1002/aja.1001410407.
- Citi, Sandra. 2018. “Intestinal Barriers Protect against Disease.” *Science* 359 (6380): 1097–98. https://doi.org/10.1126/science.aat0835.
- Darwich, Adam S., Umair Aslam, Darren M. Ashcroft, and Amin Rostami-Hodjegan. 2014. “Meta-Analysis of the Turnover of Intestinal Epithelia in Preclinical Animal Species and Humans.” *Drug Metabolism and Disposition* 42 (12): 2016–22. https://doi.org/10.1124/dmd.114.058404.
- Davis, Hayley, Shazia Irshad, Mukesh Bansal, Hannah Rafferty, Tatjana Boitsova, Chiara Bardella, Emma Jaeger, et al. 2015. “Aberrant Epithelial GREM1 Expression Initiates Colonic Tumorigenesis from Cells Outside the Stem Cell Niche.” *Nature Medicine* 21 (1): 62–70. https://doi.org/10.1038/nm.3750.
- Dijke, Peter ten, and Caroline S. Hill. 2004. “New Insights into TGF-β–Smad Signalling.” *Trends in Biochemical Sciences* 29 (5): 265–73. https://doi.org/10.1016/j.tibs.2004.03.008.
- Dupont, Sirio, Leonardo Morsut, Mariaceleste Aragona, Elena Enzo, Stefano Giulitti, Michelangelo Cordenonsi, Francesca Zanconato, et al. 2011. “Role of YAP/TAZ in Mechanotransduction.” *Nature* 474 (7350): 179–83. https://doi.org/10.1038/nature10137.
- Eckes, Beate, Roswitha Nischt, and Thomas Krieg. 2010. “Cell-Matrix Interactions in Dermal Repair and Scarring.” *Fibrogenesis & Tissue Repair* 3 (March): 4. https://doi.org/10.1186/1755-1536-3-4.
- Eming, Sabine A., Thomas Krieg, and Jeffrey M. Davidson. 2007. “Inflammation in Wound Repair: Molecular and Cellular Mechanisms.” *Journal of Investigative Dermatology* 127 (3): 514–25. https://doi.org/10.1038/sj.jid.5700701.
- Engler, Adam J., Shamik Sen, H. Lee Sweeney, and Dennis E. Discher. 2006. “Matrix Elasticity Directs Stem Cell Lineage Specification.” *Cell* 126 (4): 677–89. https://doi.org/10.1016/j.cell.2006.06.044.
- Eyden, Brian, Alan Curry, and Guofeng Wang. 2011. “Stromal Cells in the Human Gut Show Ultrastructural Features of Fibroblasts and Smooth Muscle Cells but Not Myofibroblasts.” *Journal of Cellular and Molecular Medicine* 15 (7): 1483–91. https://doi.org/10.1111/j.1582-4934.2010.01132.x.
- Fernández-Majada, Vanesa, Jordi Comelles, Verónica Acevedo, Aina Abad-Lázaro, Xavier Hernando-Momblona, Eduard Batlle, and Elena Martinez. 2021. “Subepithelial Myofibroblasts Are Critical Regulators of Intestinal Epithelial Restoration.” bioRxiv. https://doi.org/10.1101/2021.05.28.446131.
- Fevr, Tea, Sylvie Robine, Daniel Louvard, and Joerg Huelsken. 2007. “Wnt/Beta-Catenin Is Essential for Intestinal Homeostasis and Maintenance of Intestinal Stem Cells.” *Molecular and Cellular Biology* 27 (21): 7551–59. https://doi.org/10.1128/MCB.01034-07.
- Frevert, Charles W., Jessica Felgenhauer, Malgorzata Wygrecka, Madalina V. Nastase, and Liliana Schaefer. 2018. “Danger-Associated Molecular Patterns Derived From the Extracellular Matrix Provide Temporal Control of Innate Immunity.” *The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society* 66 (4): 213–27. https://doi.org/10.1369/0022155417740880.
- Friedrich, Matthias, Mathilde Pohin, Matthew A. Jackson, Ilya Korsunsky, Samuel J. Bullers, Kevin Rue-Albrecht, Zoe Christoforidou, et al. 2021. “IL-1-Driven Stromal–Neutrophil Interactions Define a Subset of Patients with Inflammatory Bowel Disease That Does Not Respond to Therapies.” *Nature Medicine* 27 (11): 1970–81. https://doi.org/10.1038/s41591-021-01520-5.
- Gaengel, Konstantin, Guillem Genové, Annika Armulik, and Christer Betsholtz. 2009. “Endothelial-Mural Cell Signaling in Vascular Development and Angiogenesis.” *Arteriosclerosis, Thrombosis, and Vascular Biology* 29 (5): 630–38. https://doi.org/10.1161/ATVBAHA.107.161521.
- Gattazzo, Francesca, Anna Urciuolo, and Paolo Bonaldo. 2014. “Extracellular Matrix: A Dynamic Microenvironment for Stem Cell Niche.” *Biochimica et Biophysica Acta* 1840 (8): 2506–19. https://doi.org/10.1016/j.bbagen.2014.01.010.
- Gehart, Helmuth, and Hans Clevers. 2019. “Tales from the Crypt: New Insights into Intestinal Stem Cells.” *Nature Reviews Gastroenterology & Hepatology* 16 (1): 19–34. https://doi.org/10.1038/s41575-018-0081-y.
- Gelbmann, C. M., S. Mestermann, V. Gross, M. Köllinger, J. Schölmerich, and W. Falk. 1999. “Strictures in Crohn’s Disease Are Characterised by an Accumulation of Mast Cells Colocalised with Laminin but Not with Fibronectin or Vitronectin.” *Gut* 45 (2): 210–17. https://doi.org/10.1136/gut.45.2.210.
- Gerbe, François, Emmanuelle Sidot, Danielle J. Smyth, Makoto Ohmoto, Ichiro Matsumoto, Valérie Dardalhon, Pierre Cesses, et al. 2016. “Intestinal Epithelial Tuft Cells Initiate Type 2 Mucosal Immunity to Helminth Parasites.” *Nature* 529 (7585): 226–30. https://doi.org/10.1038/nature16527.
- Gibson, Deanna L., Marinieve Montero, Mark J. Ropeleski, Kirk S. B. Bergstrom, Caixia Ma, Sanjoy Ghosh, Helen Merkens, et al. 2010. “Interleukin-11 Reduces TLR4-Induced Colitis in TLR2-Deficient

Mice and Restores Intestinal STAT3 Signaling." *Gastroenterology* 139 (4): 1277–88. https://doi.org/10.1053/j.gastro.2010.06.057.
- Giobbe, Giovanni Giuseppe, Claire Crowley, Camilla Luni, Sara Campinoti, Moustafa Khedr, Kai Kretzschmar, Martina Maria De Santis, et al. 2019. "Extracellular Matrix Hydrogel Derived from Decellularized Tissues Enables Endodermal Organoid Culture." *Nature Communications* 10 (1): 5658. https://doi.org/10.1038/s41467-019-13605-4.
- Giuffrida, Paolo, Marco Curti, Walid Al-Akkad, Carin Biel, Claire Crowley, Luca Frenguelli, Andrea Telese, et al. 2019. "Decellularized Human Gut as a Natural 3D Platform for Research in Intestinal Fibrosis." *Inflammatory Bowel Diseases* 25 (11): 1740–50. https://doi.org/10.1093/ibd/izz115.
- Gregorieff, Alex, Daniel Pinto, Harry Begthel, Olivier Destrée, Menno Kielman, and Hans Clevers. 2005. "Expression Pattern of Wnt Signaling Components in the Adult Intestine." *Gastroenterology* 129 (2): 626–38. https://doi.org/10.1053/j.gastro.2005.06.007.
- Greicius, Gediminas, Zahra Kabiri, Kristmundur Sigmundsson, Chao Liang, Ralph Bunte, Manvendra K. Singh, and David M. Virshup. 2018. "PDGFRα+ Pericryptal Stromal Cells Are the Critical Source of Wnts and RSPO3 for Murine Intestinal Stem Cells in Vivo." *Proceedings of the National Academy of Sciences* 115 (14): E3173–81. https://doi.org/10.1073/pnas.1713510115.
- Gribble, Fiona M., and Frank Reimann. 2019. "Function and Mechanisms of Enteroendocrine Cells and Gut Hormones in Metabolism." *Nature Reviews Endocrinology* 15 (4): 226–37. https://doi.org/10.1038/s41574-019-0168-8.
- Hageman, Joris H., Maria C. Heinz, Kai Kretzschmar, Jelte van der Vaart, Hans Clevers, and Hugo J. G. Snippert. 2020. "Intestinal Regeneration: Regulation by the Microenvironment." *Developmental Cell* 54 (4): 435–46. https://doi.org/10.1016/j.devcel.2020.07.009.
- Hao, Huai-Xiang, Yang Xie, Yue Zhang, Olga Charlat, Emma Oster, Monika Avello, Hong Lei, et al. 2012. "ZNRF3 Promotes Wnt Receptor Turnover in an R-Spondin-Sensitive Manner." *Nature* 485 (7397): 195–200. https://doi.org/10.1038/nature11019.
- Hao, Jin, Yueling Zhang, Yating Wang, Rui Ye, Jingyi Qiu, Zhihe Zhao, and Juan Li. 2014. "Role of Extracellular Matrix and YAP/TAZ in Cell Fate Determination." *Cellular Signalling* 26 (2): 186–91. https://doi.org/10.1016/j.cellsig.2013.11.006.
- Haramis, Anna-Pavlina G., Harry Begthel, Maaike van den Born, Johan van Es, Suzanne Jonkheer, G. Johan A. Offerhaus, and Hans Clevers. 2004. "De Novo Crypt Formation and Juvenile Polyposis on BMP Inhibition in Mouse Intestine." *Science (New York, N.Y.)* 303 (5664): 1684–86. https://doi.org/10.1126/science.1093587.
- Hardwick, James C. H., Gijs R. Van Den Brink, Sylvia A. Bleuming, Isabel Ballester, Jan M. H. Van Den Brande, Josbert J. Keller, G. Johan A. Offerhaus, Sander J. H. Van Deventer, and Maikel P. Peppelenbosch. 2004. "Bone Morphogenetic Protein 2 Is Expressed by, and Acts upon, Mature Epithelial Cells in the Colon." *Gastroenterology* 126 (1): 111–21. https://doi.org/10.1053/j.gastro.2003.10.067.
- Helander, Herbert F, and Lars Fändriks. 2014. "Surface Area of the Digestive Tract – Revisited." *Scandinavian Journal of Gastroenterology* 49 (6): 681–89. https://doi.org/10.3109/00365521.2014.898326.
- Hinz, B., P. Pittet, J. Smith-Clerc, C. Chaponnier, and J.-J. Meister. 2004. "Myofibroblast Development Is Characterized by Specific Cell-Cell Adherens Junctions." *Molecular Biology of the Cell* 15 (9): 4310–20. https://doi.org/10.1091/mbc.e04-05-0386.
- Howitt, Michael R., Sydney Lavoie, Monia Michaud, Arthur M. Blum, Sara V. Tran, Joel V. Weinstock, Carey Ann Gallini, et al. 2016. "Tuft Cells, Taste-Chemosensory Cells, Orchestrate Parasite Type 2 Immunity in the Gut." *Science* 351 (6279): 1329–33. https://doi.org/10.1126/science.aaf1648.
- Humphries, Jonathan D., Adam Byron, and Martin J. Humphries. 2006. "Integrin Ligands at a Glance." *Journal of Cell Science* 119 (19): 3901–3. https://doi.org/10.1242/jcs.03098.
- Hynes, Richard O. 2002. "Integrins: Bidirectional, Allosteric Signaling Machines." *Cell* 110 (6): 673–87. https://doi.org/10.1016/S0092-8674(02)00971-6.
- ———. 2009. "The Extracellular Matrix: Not Just Pretty Fibrils." *Science (New York, N.Y.)* 326 (5957): 1216–19. https://doi.org/10.1126/science.1176009.
- Iozzo, R. V. 1998. "Matrix Proteoglycans: From Molecular Design to Cellular Function." *Annual Review of Biochemistry* 67: 609–52. https://doi.org/10.1146/annurev.biochem.67.1.609.
- Iqbal, Sharif, Simon Andersson, Ernesta Nestaite, Nalle Pentinmikko, Ashish Kumar, Daniel Borshagovski, Anna Webb, et al. 2021. "Fetal-like Reversion in the Regenerating Intestine Is Regulated by Mesenchymal Asporin." bioRxiv. https://doi.org/10.1101/2021.06.24.449590.

- Ireland, Heather, Richard Kemp, Carol Houghton, Louise Howard, Alan R. Clarke, Owen J. Sansom, and Douglas J. Winton. 2004. "Inducible Cre-Mediated Control of Gene Expression in the Murine Gastrointestinal Tract: Effect of Loss of β-Catenin." *Gastroenterology* 126 (5): 1236–46. https://doi.org/10.1053/j.gastro.2004.03.020.
- Johansson, Malin E. V., Jessica M. Holmén Larsson, and Gunnar C. Hansson. 2011. "The Two Mucus Layers of Colon Are Organized by the MUC2 Mucin, Whereas the Outer Layer Is a Legislator of Host–Microbial Interactions." *Proceedings of the National Academy of Sciences* 108 (supplement_1): 4659–65. https://doi.org/10.1073/pnas.1006451107.
- Jostins, Luke, Stephan Ripke, Rinse K. Weersma, Richard H. Duerr, Dermot P. McGovern, Ken Y. Hui, James C. Lee, et al. 2012. "Host–Microbe Interactions Have Shaped the Genetic Architecture of Inflammatory Bowel Disease." *Nature* 491 (7422): 119–24. https://doi.org/10.1038/nature11582.
- Kabiri, Zahra, Gediminas Greicius, Babita Madan, Steffen Biechele, Zhendong Zhong, Hamed Zaribafzadeh, null Edison, et al. 2014. "Stroma Provides an Intestinal Stem Cell Niche in the Absence of Epithelial Wnts." *Development (Cambridge, England)* 141 (11): 2206–15. https://doi.org/10.1242/dev.104976.
- Kaiko, Gerard E., Stacy H. Ryu, Olivia I. Koues, Patrick L. Collins, Lilianna Solnica-Krezel, Edward J. Pearce, Erika L. Pearce, Eugene M. Oltz, and Thaddeus S. Stappenbeck. 2016. "The Colonic Crypt Protects Stem Cells from Microbiota-Derived Metabolites." *Cell* 165 (7): 1708–20. https://doi.org/10.1016/j.cell.2016.05.018.
- Kalluri, Raghu. 2016. "The Biology and Function of Fibroblasts in Cancer." *Nature Reviews Cancer* 16 (9): 582–98. https://doi.org/10.1038/nrc.2016.73.
- Karpus, Olga N., B. Florien Westendorp, Jacqueline L. M. Vermeulen, Sander Meisner, Jan Koster, Vanesa Muncan, Manon E. Wildenberg, and Gijs R. van den Brink. 2019. "Colonic CD90+ Crypt Fibroblasts Secrete Semaphorins to Support Epithelial Growth." *Cell Reports* 26 (13): 3698-3708.e5. https://doi.org/10.1016/j.celrep.2019.02.101.
- Karsdal, M. A., S. H. Nielsen, D. J. Leeming, L. L. Langholm, M. J. Nielsen, T. Manon-Jensen, A. Siebuhr, et al. 2017. "The Good and the Bad Collagens of Fibrosis - Their Role in Signaling and Organ Function." *Advanced Drug Delivery Reviews* 121 (November): 43–56. https://doi.org/10.1016/j.addr.2017.07.014.
- Kessenbrock, Kai, Vicki Plaks, and Zena Werb. 2010. "Matrix Metalloproteinases: Regulators of the Tumor Microenvironment." *Cell* 141 (1): 52–67. https://doi.org/10.1016/j.cell.2010.03.015.
- Kim, Kyung-Ah, Jingsong Zhao, Susan Andarmani, Makoto Kakitani, Takeshi Oshima, Minke E. Binnerts, Arie Abo, Kazuma Tomizuka, and Walter D. Funk. 2006. "R-Spondin Proteins: A Novel Link to Beta-Catenin Activation." *Cell Cycle (Georgetown, Tex.)* 5 (1): 23–26. https://doi.org/10.4161/cc.5.1.2305.
- Kim, Suran, Sungjin Min, Yi Sun Choi, Sung-Hyun Jo, Jae Hun Jung, Kyusun Han, Jin Kim, et al. 2022. "Tissue Extracellular Matrix Hydrogels as Alternatives to Matrigel for Culturing Gastrointestinal Organoids." *Nature Communications* 13 (1): 1692. https://doi.org/10.1038/s41467-022-29279-4.
- Kinchen, James, Hannah H. Chen, Kaushal Parikh, Agne Antanaviciute, Marta Jagielowicz, David Fawkner-Corbett, Neil Ashley, et al. 2018. "Structural Remodeling of the Human Colonic Mesenchyme in Inflammatory Bowel Disease." *Cell* 175 (2): 372-386.e17. https://doi.org/10.1016/j.cell.2018.08.067.
- Knoop, Kathryn A., Nachiket Kumar, Betsy R. Butler, Senthilkumar K. Sakthivel, Rebekah T. Taylor, Tomonori Nochi, Hisaya Akiba, Hideo Yagita, Hiroshi Kiyono, and Ifor R. Williams. 2009. "RANKL Is Necessary and Sufficient to Initiate Development of Antigen-Sampling M Cells in the Intestinal Epithelium1." *The Journal of Immunology* 183 (9): 5738–47. https://doi.org/10.4049/jimmunol.0901563.
- Knoop, Kathryn A., and Rodney D. Newberry. 2018. "Goblet Cells: Multifaceted Players in Immunity at Mucosal Surfaces." *Mucosal Immunology* 11 (6): 1551–57. https://doi.org/10.1038/s41385-018-0039-y.
- Kobori, Ayako, Yuhki Yagi, Hirotsugu Imaeda, Hiromitsu Ban, Shigeki Bamba, Tomoyuki Tsujikawa, Yasuharu Saito, Yoshihide Fujiyama, and Akira Andoh. 2010. "Interleukin-33 Expression Is Specifically Enhanced in Inflamed Mucosa of Ulcerative Colitis." *Journal of Gastroenterology* 45 (10): 999–1007. https://doi.org/10.1007/s00535-010-0245-1.
- Koliaraki, Vasiliki, Manolis Pasparakis, and George Kollias. 2015. "IKKβ in Intestinal Mesenchymal Cells Promotes Initiation of Colitis-Associated Cancer." *Journal of Experimental Medicine* 212 (13): 2235–51. https://doi.org/10.1084/jem.20150542.
- Koliaraki, Vasiliki, Alejandro Prados, Marietta Armaka, and George Kollias. 2020. "The Mesenchymal Context in Inflammation, Immunity and Cancer." *Nature Immunology* 21 (9): 974–82. https://doi.org/10.1038/s41590-020-0741-2.

- Krausova, Michaela, and Vladimir Korinek. 2014. “Wnt Signaling in Adult Intestinal Stem Cells and Cancer.” *Cellular Signalling* 26 (3): 570–79. https://doi.org/10.1016/j.cellsig.2013.11.032.
- Kuhnert, Frank, Corrine R. Davis, Hsiao-Ting Wang, Pauline Chu, Mark Lee, Jenny Yuan, Roel Nusse, and Calvin J. Kuo. 2004. “Essential Requirement for Wnt Signaling in Proliferation of Adult Small Intestine and Colon Revealed by Adenoviral Expression of Dickkopf-1.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (1): 266–71. https://doi.org/10.1073/pnas.2536800100.
- Kurahashi, Masaaki, Yasuko Nakano, Lauren E. Peri, Jared B. Townsend, Sean M. Ward, and Kenton M. Sanders. 2013. “A Novel Population of Subepithelial Platelet-Derived Growth Factor Receptor α-Positive Cells in the Mouse and Human Colon.” *American Journal of Physiology-Gastrointestinal and Liver Physiology* 304 (9): G823–34. https://doi.org/10.1152/ajpgi.00001.2013.
- Latella, Giovanni, Gerhard Rogler, Giorgos Bamias, Christine Breynaert, Jon Florholmen, Gianluca Pellino, Shimon Reif, Speca Silvia, and Ian C. Lawrance. 2014. “Results of the 4th Scientific Workshop of the ECCO (I): Pathophysiology of Intestinal Fibrosis in IBD.” *Journal of Crohn’s and Colitis* 8 (10): 1147–65. https://doi.org/10.1016/j.crohns.2014.03.008.
- Laukoetter, Mike G., Porfirio Nava, Winston Y. Lee, Eric A. Severson, Christopher T. Capaldo, Brian A. Babbin, Ifor R. Williams, et al. 2007. “JAM-A Regulates Permeability and Inflammation in the Intestine in Vivo.” *The Journal of Experimental Medicine* 204 (13): 3067–76. https://doi.org/10.1084/jem.20071416.
- Lebbink, Robert Jan, Nicolas Raynal, Talitha de Ruiter, Dominique G. Bihan, Richard W. Farndale, and Linde Meyaard. 2009. “Identification of Multiple Potent Binding Sites for Human Leukocyte Associated Ig-like Receptor LAIR on Collagens II and III.” *Matrix Biology: Journal of the International Society for Matrix Biology* 28 (4): 202–10. https://doi.org/10.1016/j.matbio.2009.03.005.
- Lebbink, Robert Jan, Talitha de Ruiter, Jelle Adelmeijer, Arjan B. Brenkman, Joop M. van Helvoort, Manuel Koch, Richard W. Farndale, et al. 2006. “Collagens Are Functional, High Affinity Ligands for the Inhibitory Immune Receptor LAIR-1.” *Journal of Experimental Medicine* 203 (6): 1419–25. https://doi.org/10.1084/jem.20052554.
- Lechuga, Susana, Manuel B. Braga-Neto, Nayden G. Naydenov, Florian Rieder, and Andrei I. Ivanov. 2023. “Understanding Disruption of the Gut Barrier during Inflammation: Should We Abandon Traditional Epithelial Cell Lines and Switch to Intestinal Organoids?” *Frontiers in Immunology* 14 (February): 1108289. https://doi.org/10.3389/fimmu.2023.1108289.
- Li, Chao, and John F. Kuemmerle. 2014. “Mechanisms That Mediate the Development of Fibrosis in Patients With Crohn’s Disease.” *Inflammatory Bowel Diseases* 20 (7): 1250–58. https://doi.org/10.1097/MIB.0000000000000043.
- Li, Hui Joyce, Archana Kapoor, Maryann Giel-Moloney, Guido Rindi, and Andrew B. Leiter. 2012. “Notch Signaling Differentially Regulates the Cell Fate of Early Endocrine Precursor Cells and Their Maturing Descendants in the Mouse Pancreas and Intestine.” *Developmental Biology* 371 (2): 156–69. https://doi.org/10.1016/j.ydbio.2012.08.023.
- Li, Linheng, and Hans Clevers. 2010. “Coexistence of Quiescent and Active Adult Stem Cells in Mammals.” *Science (New York, N.Y.)* 327 (5965): 542–45. https://doi.org/10.1126/science.1180794.
- Lindahl, Per, Bengt R. Johansson, Per Levéen, and Christer Betsholtz. 1997. “Pericyte Loss and Microaneurysm Formation in PDGF-B-Deficient Mice.” *Science* 277 (5323): 242–45. https://doi.org/10.1126/science.277.5323.242.
- Lu, Pengfei, Valerie M. Weaver, and Zena Werb. 2012. “The Extracellular Matrix: A Dynamic Niche in Cancer Progression.” *Journal of Cell Biology* 196 (4): 395–406. https://doi.org/10.1083/jcb.201102147.
- Mabbott, Neil A., David S. Donaldson, Hiroshi Ohno, Ifor R. Williams, and Arvind Mahajan. 2013. “Microfold (M) Cells: Important Immunosurveillance Posts in the Intestinal Epithelium.” *Mucosal Immunology* 6 (4): 666–77. https://doi.org/10.1038/mi.2013.30.
- Mahapatro, Mousumi, Sebastian Foersch, Manuela Hefele, Gui-Wei He, Elisa Giner-Ventura, Tamar Mchedlidze, Markus Kindermann, et al. 2016. “Programming of Intestinal Epithelial Differentiation by IL-33 Derived from Pericryptal Fibroblasts in Response to Systemic Infection.” *Cell Reports* 15 (8): 1743–56. https://doi.org/10.1016/j.celrep.2016.04.049.
- Marchelletta, Ronald R., Moorthy Krishnan, Marianne R. Spalinger, Taylaur W. Placone, Rocio Alvarez, Anica Sayoc-Becerra, Vinicius Canale, et al. n.d. “T Cell Protein Tyrosine Phosphatase Protects Intestinal Barrier Function by Restricting Epithelial Tight Junction Remodeling.” *The Journal of Clinical Investigation* 131 (17): e138230. https://doi.org/10.1172/JCI138230.
- Martín-Alonso, Mara, Sharif Iqbal, Pia M. Vornewald, Håvard T. Lindholm, Mirjam J. Damen, Fernando Martínez, Sigrid Hoel, et al. 2021. “Smooth Muscle-Specific MMP17 (MT4-MMP) Regulates the Intestinal

Stem Cell Niche and Regeneration after Damage." *Nature Communications* 12 (1): 6741. https://doi.org/10.1038/s41467-021-26904-6.
- Massagué, Joan. 2012. "TGFβ Signalling in Context." *Nature Reviews. Molecular Cell Biology* 13 (10): 616–30. https://doi.org/10.1038/nrm3434.
- Mayorca-Guiliani, Alejandro E., Oliver Willacy, Chris D. Madsen, Maria Rafaeva, Stefanie Elisabeth Heumüller, Felix Bock, Gerhard Sengle, et al. 2019. "Decellularization and Antibody Staining of Mouse Tissues to Map Native Extracellular Matrix Structures in 3D." *Nature Protocols* 14 (12): 3395–3425. https://doi.org/10.1038/s41596-019-0225-8.
- McDole, Jeremiah R., Leroy W. Wheeler, Keely G. McDonald, Baomei Wang, Vjollca Konjufca, Kathryn A. Knoop, Rodney D. Newberry, and Mark J. Miller. 2012. "Goblet Cells Deliver Luminal Antigen to CD103+ Dendritic Cells in the Small Intestine." *Nature* 483 (7389): 345–49. https://doi.org/10.1038/nature10863.
- McMahon, Meagan, Siying Ye, Jess Pedrina, Daniel Dlugolenski, and John Stambas. 2021. "Extracellular Matrix Enzymes and Immune Cell Biology." *Frontiers in Molecular Biosciences* 8. https://www.frontiersin.org/articles/10.3389/fmolb.2021.703868.
- Meran, Laween, Isobel Massie, Sara Campinoti, Anne E. Weston, Riana Gaifulina, Lucinda Tullie, Peter Faull, et al. 2020. "Engineering Transplantable Jejunal Mucosal Grafts Using Patient-Derived Organoids from Children with Intestinal Failure." *Nature Medicine* 26 (10): 1593–1601. https://doi.org/10.1038/s41591-020-1024-z.
- Meyaard, Linde. 2010. "LAIR and Collagens in Immune Regulation." *Immunology Letters* 128 (1): 26–28. https://doi.org/10.1016/j.imlet.2009.09.014.
- Mizoguchi, Atsushi, Takahito Takeuchi, Hidetomo Himuro, Toshiyuki Okada, and Emiko Mizoguchi. 2016. "Genetically Engineered Mouse Models for Studying Inflammatory Bowel Disease." *The Journal of Pathology* 238 (2): 205–19. https://doi.org/10.1002/path.4640.
- Moltke, Jakob von, Ming Ji, Hong-Erh Liang, and Richard M. Locksley. 2016. "Tuft-Cell-Derived IL-25 Regulates an Intestinal ILC2–Epithelial Response Circuit." *Nature* 529 (7585): 221–25. https://doi.org/10.1038/nature16161.
- Monteleone, Ivan, Massimo Federici, Massimiliano Sarra, Eleonora Franzè, Viviana Casagrande, Francesca Zorzi, Michele Cavalera, et al. 2012. "Tissue Inhibitor of Metalloproteinase-3 Regulates Inflammation in Human and Mouse Intestine." *Gastroenterology* 143 (5): 1277-1287.e4. https://doi.org/10.1053/j.gastro.2012.07.016.
- Montgomery, Robert K., Diana L. Carlone, Camilla A. Richmond, Loredana Farilla, Mariette E. G. Kranendonk, Daniel E. Henderson, Nana Yaa Baffour-Awuah, et al. 2011. "Mouse Telomerase Reverse Transcriptase (MTert) Expression Marks Slowly Cycling Intestinal Stem Cells." *Proceedings of the National Academy of Sciences* 108 (1): 179–84. https://doi.org/10.1073/pnas.1013004108.
- Mörbe, Urs M., Peter B. Jørgensen, Thomas M. Fenton, Nicole von Burg, Lene B. Riis, Jo Spencer, and William W. Agace. 2021. "Human Gut-Associated Lymphoid Tissues (GALT); Diversity, Structure, and Function." *Mucosal Immunology* 14 (4): 793–802. https://doi.org/10.1038/s41385-021-00389-4.
- Muncie, Jonathon M., and Valerie M. Weaver. 2018. "The Physical and Biochemical Properties of the Extracellular Matrix Regulate Cell Fate." *Current Topics in Developmental Biology* 130: 1–37. https://doi.org/10.1016/bs.ctdb.2018.02.002.
- Naba, Alexandra, Karl R. Clauser, Sebastian Hoersch, Hui Liu, Steven A. Carr, and Richard O. Hynes. 2012. "The Matrisome: In Silico Definition and in Vivo Characterization by Proteomics of Normal and Tumor Extracellular Matrices." *Molecular & Cellular Proteomics: MCP* 11 (4): M111.014647. https://doi.org/10.1074/mcp.M111.014647.
- O'Sullivan, Shane, John F. Gilmer, and Carlos Medina. 2015. "Matrix Metalloproteinases in Inflammatory Bowel Disease: An Update." *Mediators of Inflammation* 2015: 964131. https://doi.org/10.1155/2015/964131.
- Page-McCaw, Andrea, Andrew J. Ewald, and Zena Werb. 2007. "Matrix Metalloproteinases and the Regulation of Tissue Remodelling." *Nature Reviews Molecular Cell Biology* 8 (3): 221–33. https://doi.org/10.1038/nrm2125.
- Park, Ji Min, Jeongseok Kim, Yoo Jin Lee, Sung Uk Bae, and Hye Won Lee. 2023. "Inflammatory Bowel Disease–Associated Intestinal Fibrosis." *Journal of Pathology and Translational Medicine* 57 (1): 60–66. https://doi.org/10.4132/jptm.2022.11.02.
- Pathak, Medha M., Jamison L. Nourse, Truc Tran, Jennifer Hwe, Janahan Arulmoli, Dai Trang T. Le, Elena Bernardis, Lisa A. Flanagan, and Francesco Tombola. 2014. "Stretch-Activated Ion Channel Piezo1 Directs

Lineage Choice in Human Neural Stem Cells." *Proceedings of the National Academy of Sciences of the United States of America* 111 (45): 16148–53. https://doi.org/10.1073/pnas.1409802111.

- Peng, Weng Chuan, Wim de Lau, Pramod K. Madoori, Federico Forneris, Joke C. M. Granneman, Hans Clevers, and Piet Gros. 2013. "Structures of Wnt-Antagonist ZNRF3 and Its Complex with R-Spondin 1 and Implications for Signaling." *PLoS ONE* 8 (12): e83110. https://doi.org/10.1371/journal.pone.0083110.
- Perez-Shibayama, Christian, Cristina Gil-Cruz, and Burkhard Ludewig. 2019. "Fibroblastic Reticular Cells at the Nexus of Innate and Adaptive Immune Responses." *Immunological Reviews* 289 (1): 31–41. https://doi.org/10.1111/imr.12748.
- Peterson, Lance W., and David Artis. 2014. "Intestinal Epithelial Cells: Regulators of Barrier Function and Immune Homeostasis." *Nature Reviews Immunology* 14 (3): 141–53. https://doi.org/10.1038/nri3608.
- Petrey, Aaron C., and Carol A. de la Motte. 2017. "The Extracellular Matrix in IBD: A Dynamic Mediator of Inflammation." *Current Opinion in Gastroenterology* 33 (4): 234–38. https://doi.org/10.1097/MOG.0000000000000368.
- Pinchuk, I. V., R. C. Mifflin, J. I. Saada, and D. W. Powell. 2010. "Intestinal Mesenchymal Cells." *Current Gastroenterology Reports* 12 (5): 310–18. https://doi.org/10.1007/s11894-010-0135-y.
- Pinto, Daniel, Alex Gregorieff, Harry Begthel, and Hans Clevers. 2003. "Canonical Wnt Signals Are Essential for Homeostasis of the Intestinal Epithelium." *Genes & Development* 17 (14): 1709–13. https://doi.org/10.1101/gad.267103.
- Plutoni, Cédric, Sarah Keil, Carlos Zeledon, Lara Elis Alberici Delsin, Barbara Decelle, Philippe P. Roux, Sébastien Carréno, and Gregory Emery. 2019. "Misshapen Coordinates Protrusion Restriction and Actomyosin Contractility during Collective Cell Migration." *Nature Communications* 10 (September): 3940. https://doi.org/10.1038/s41467-019-11963-7.
- Pompili, Simona, Giovanni Latella, Eugenio Gaudio, Roberta Sferra, and Antonella Vetuschi. 2021. "The Charming World of the Extracellular Matrix: A Dynamic and Protective Network of the Intestinal Wall." *Frontiers in Medicine* 8. https://www.frontiersin.org/articles/10.3389/fmed.2021.610189.
- Porter, E.M., C.L. Bevins, D. Ghosh, and T. Ganz. 2002. "The Multifaceted Paneth Cell." *Cellular and Molecular Life Sciences CMLS* 59 (1): 156–70. https://doi.org/10.1007/s00018-002-8412-z.
- Potten, C.S., and M. Loeffler. 1990. "Stem Cells: Attributes, Cycles, Spirals, Pitfalls and Uncertainties Lessons for and from the Crypt." *Development* 110 (4): 1001–20. https://doi.org/10.1242/dev.110.4.1001.
- Powell, D.W., I.V. Pinchuk, J.I. Saada, Xin Chen, and R.C. Mifflin. 2011. "Mesenchymal Cells of the Intestinal Lamina Propria." *Annual Review of Physiology* 73 (1): 213–37. https://doi.org/10.1146/annurev.physiol.70.113006.100646.
- Rawat, Parkash Singh, Ahkam Saddam Seyed Hameed, Xiangfeng Meng, and Weifeng Liu. 2022. "Utilization of Glycosaminoglycans by the Human Gut Microbiota: Participating Bacteria and Their Enzymatic Machineries." *Gut Microbes* 14 (1): 2068367. https://doi.org/10.1080/19490976.2022.2068367.
- Rieder, Florian, Sean P. Kessler, Gail A. West, Shardul Bhilocha, Carol de la Motte, Tammy M. Sadler, Banu Gopalan, Eleni Stylianou, and Claudio Fiocchi. 2011. "Inflammation-Induced Endothelial-to-Mesenchymal Transition: A Novel Mechanism of Intestinal Fibrosis." *The American Journal of Pathology* 179 (5): 2660–73. https://doi.org/10.1016/j.ajpath.2011.07.042.
- Rogler, G., C. M. Gelbmann, D. Vogl, M. Brunner, J. Schölmerich, W. Falk, T. Andus, and K. Brand. 2001. "Differential Activation of Cytokine Secretion in Primary Human Colonic Fibroblast/Myofibroblast Cultures." *Scandinavian Journal of Gastroenterology* 36 (4): 389–98. https://doi.org/10.1080/003655201300051216.
- Roulis, Manolis, Christoforos Nikolaou, Elena Kotsaki, Eleanna Kaffe, Niki Karagianni, Vasiliki Koliaraki, Klelia Salpea, et al. 2014. "Intestinal Myofibroblast-Specific Tpl2-Cox-2-PGE2 Pathway Links Innate Sensing to Epithelial Homeostasis." *Proceedings of the National Academy of Sciences* 111 (43): E4658–67. https://doi.org/10.1073/pnas.1415762111.
- Sacchetti, Benedetto, Alessia Funari, Cristina Remoli, Giuseppe Giannicola, Gesine Kogler, Stefanie Liedtke, Giulio Cossu, et al. 2016. "No Identical 'Mesenchymal Stem Cells' at Different Times and Sites: Human Committed Progenitors of Distinct Origin and Differentiation Potential Are Incorporated as Adventitial Cells in Microvessels." *Stem Cell Reports* 6 (6): 897–913. https://doi.org/10.1016/j.stemcr.2016.05.011.
- Sancho, Rocio, Catherine A Cremona, and Axel Behrens. 2015. "Stem Cell and Progenitor Fate in the Mammalian Intestine: Notch and Lateral Inhibition in Homeostasis and Disease." *EMBO Reports* 16 (5): 571–81. https://doi.org/10.15252/embr.201540188.

- Sanders, Kenton M., Sean M. Ward, and Sang Don Koh. 2014. “Interstitial Cells: Regulators of Smooth Muscle Function.” *Physiological Reviews* 94 (3): 859–907. https://doi.org/10.1152/physrev.00037.2013.
- Sangiorgi, Eugenio, and Mario R. Capecchi. 2008. “Bmi1 Is Expressed in Vivo in Intestinal Stem Cells.” *Nature Genetics* 40 (7): 915–20. https://doi.org/10.1038/ng.165.
- Sato, Toshiro, Johan H. van Es, Hugo J. Snippert, Daniel E. Stange, Robert G. Vries, Maaike van den Born, Nick Barker, Noah F. Shroyer, Marc van de Wetering, and Hans Clevers. 2011. “Paneth Cells Constitute the Niche for Lgr5 Stem Cells in Intestinal Crypts.” *Nature* 469 (7330): 415–18. https://doi.org/10.1038/nature09637.
- Schultz, Gregory S., and Annette Wysocki. 2009. “Interactions between Extracellular Matrix and Growth Factors in Wound Healing.” *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society* 17 (2): 153–62. https://doi.org/10.1111/j.1524-475X.2009.00466.x.
- Schweizer, Liang, and Harold Varmus. 2003. “Wnt/Wingless Signaling through β-Catenin Requires the Function of Both LRP/Arrow and Frizzled Classes of Receptors.” *BMC Cell Biology* 4 (May): 4. https://doi.org/10.1186/1471-2121-4-4.
- Signore, Michele, Anna Maria Cerio, Alessandra Boe, Alfredo Pagliuca, Valentina Zaottini, Ilaria Schiavoni, Giorgio Fedele, et al. 2012. “Identity and Ranking of Colonic Mesenchymal Stromal Cells.” *Journal of Cellular Physiology* 227 (9): 3291–3300. https://doi.org/10.1002/jcp.24027.
- Singh, Udai P., Narendra P. Singh, E. Angela Murphy, Robert L. Price, Raja Fayad, Mitzi Nagarkatti, and Prakash S. Nagarkatti. 2016. “Chemokine and Cytokine Levels in Inflammatory Bowel Disease Patients.” *Cytokine* 77 (January): 44–49. https://doi.org/10.1016/j.cyto.2015.10.008.
- Souza, Natalie de. 2017. “Organoid Culture.” *Nature Methods* 14 (1): 35–35. https://doi.org/10.1038/nmeth.4122.
- Speca, Silvia, Ilaria Giusti, Florian Rieder, and Giovanni Latella. 2012. “Cellular and Molecular Mechanisms of Intestinal Fibrosis.” *World Journal of Gastroenterology : WJG* 18 (28): 3635–61. https://doi.org/10.3748/wjg.v18.i28.3635.
- Sponheim, Jon, Jürgen Pollheimer, Trine Olsen, Johanna Balogh, Clara Hammarström, Tamara Loos, Monika Kasprzycka, et al. 2010. “Inflammatory Bowel Disease-Associated Interleukin-33 Is Preferentially Expressed in Ulceration-Associated Myofibroblasts.” *The American Journal of Pathology* 177 (6): 2804–15. https://doi.org/10.2353/ajpath.2010.100378.
- Steen, Emily H., Xinyi Wang, Swathi Balaji, Manish J. Butte, Paul L. Bollyky, and Sundeep G. Keswani. 2020. “The Role of the Anti-Inflammatory Cytokine Interleukin-10 in Tissue Fibrosis.” *Advances in Wound Care* 9 (4): 184–98. https://doi.org/10.1089/wound.2019.1032.
- Storm, Elaine E., Steffen Durinck, Felipe de Sousa e Melo, Jarrod Tremayne, Noelyn Kljavin, Christine Tan, Xiaofen Ye, et al. 2016. “Targeting PTPRK-RSPO3 Colon Tumours Promotes Differentiation and Loss of Stem-Cell Function.” *Nature* 529 (7584): 97–100. https://doi.org/10.1038/nature16466.
- Strup-Perrot, Carine, Marie-Catherine Vozenin-Brotons, Marie Vandamme, Marc Benderitter, and Denis Mathe. 2006. “Expression and Activation of MMP -2, -3, -9, -14 Are Induced in Rat Colon after Abdominal X-Irradiation.” *Scandinavian Journal of Gastroenterology* 41 (1): 60–70. https://doi.org/10.1080/00365520510023963.
- Stzepourginski, Igor, Giulia Nigro, Jean-Marie Jacob, Sophie Dulauroy, Philippe J. Sansonetti, Gérard Eberl, and Lucie Peduto. 2017. “CD34+ Mesenchymal Cells Are a Major Component of the Intestinal Stem Cells Niche at Homeostasis and after Injury.” *Proceedings of the National Academy of Sciences* 114 (4): E506–13. https://doi.org/10.1073/pnas.1620059114.
- Suzuki, Hideaki, Shinichi Sekine, Kosuke Kataoka, David W. Pascual, Massimo Maddaloni, Ryoki Kobayashi, Keiko Fujhashi, Haruo Kozono, Jerry R. McGhee, and Kohtaro Fujihashi. 2008. “Ovalbumin-Protein Σ1 M Cell Targeting Facilitates Oral Tolerance with Reduction of Antigen-Specific CD4+ T Cells.” *Gastroenterology* 135 (3): 917–25. https://doi.org/10.1053/j.gastro.2008.05.037.
- Takeda, Norifumi, Rajan Jain, Matthew R. LeBoeuf, Qiaohong Wang, Min Min Lu, and Jonathan A. Epstein. 2011. “Interconversion Between Intestinal Stem Cell Populations in Distinct Niches.” *Science* 334 (6061): 1420–24. https://doi.org/10.1126/science.1213214.
- Thomson, Carolyn A., Serge A. van de Pavert, Michelle Stakenborg, Evelien Labeeuw, Gianluca Matteoli, Allan McI Mowat, and Robert J. B. Nibbs. 2018. “Expression of the Atypical Chemokine Receptor ACKR4 Identifies a Novel Population of Intestinal Submucosal Fibroblasts That Preferentially Expresses Endothelial Cell Regulators.” *Journal of Immunology (Baltimore, Md.: 1950)* 201 (1): 215–29. https://doi.org/10.4049/jimmunol.1700967.

- Tian, Hua, Brian Biehs, Cecilia Chiu, Christian W. Siebel, Yan Wu, Mike Costa, Frederic J. de Sauvage, and Ophir D. Klein. 2015. "Opposing Activities of Notch and Wnt Signaling Regulate Intestinal Stem Cells and Gut Homeostasis." *Cell Reports* 11 (1): 33–42. https://doi.org/10.1016/j.celrep.2015.03.007.
- Tomlin, Hannah, and Anna M. Piccinini. 2018. "A Complex Interplay between the Extracellular Matrix and the Innate Immune Response to Microbial Pathogens." *Immunology* 155 (2): 186–201. https://doi.org/10.1111/imm.12972.
- Toyoda, H., K. Ina, H. Kitamura, T. Tsuda, and T. Shimada. 2008. "Organization of the Lamina Propria Mucosae of Rat Intestinal Mucosa, with Special Reference to the Subepithelial Connective Tissue." *Acta Anatomica* 158 (3): 172–84. https://doi.org/10.1159/000147928.
- Tu, Zhengkun, Adel Bozorgzadeh, Robert H. Pierce, Jonathan Kurtis, I. Nicholas Crispe, and Mark S. Orloff. 2008. "TLR-Dependent Cross Talk between Human Kupffer Cells and NK Cells." *Journal of Experimental Medicine* 205 (1): 233–44. https://doi.org/10.1084/jem.20072195.
- Turner, Jerrold R. 2009. "Intestinal Mucosal Barrier Function in Health and Disease." *Nature Reviews Immunology* 9 (11): 799–809. https://doi.org/10.1038/nri2653.
- Vaday, G. G., R. Hershkoviz, M. A. Rahat, N. Lahat, L. Cahalon, and O. Lider. 2000. "Fibronectin-Bound TNF-Alpha Stimulates Monocyte Matrix Metalloproteinase-9 Expression and Regulates Chemotaxis." *Journal of Leukocyte Biology* 68 (5): 737–47.
- Vaday, G. G., and O. Lider. 2000. "Extracellular Matrix Moieties, Cytokines, and Enzymes: Dynamic Effects on Immune Cell Behavior and Inflammation." *Journal of Leukocyte Biology* 67 (2): 149–59. https://doi.org/10.1002/jlb.67.2.149.
- Valtieri, Mauro, and Antonio Sorrentino. 2008. "The Mesenchymal Stromal Cell Contribution to Homeostasis." *Journal of Cellular Physiology* 217 (2): 296–300. https://doi.org/10.1002/jcp.21521.
- Vidrich, Alda, Jenny M. Buzan, Brooks Brodrick, Chibuzo Ilo, Leigh Bradley, Kirstin Skaar Fendig, Thomas Sturgill, and Steven M. Cohn. 2009. "Fibroblast Growth Factor Receptor-3 Regulates Paneth Cell Lineage Allocation and Accrual of Epithelial Stem Cells during Murine Intestinal Development." *American Journal of Physiology - Gastrointestinal and Liver Physiology* 297 (1): G168–78. https://doi.org/10.1152/ajpgi.90589.2008.
- Walma, David A. Cruz, and Kenneth M. Yamada. 2020. "The Extracellular Matrix in Development." *Development (Cambridge, England)* 147 (10): dev175596. https://doi.org/10.1242/dev.175596.
- Walter, Romina J., Steffen J. Sonnentag, Leonel Munoz-Sagredo, Melanie Merkel, Ludovic Richert, Felix Bunert, Yvonne M. Heneka, et al. 2022. "Wnt Signaling Is Boosted during Intestinal Regeneration by a CD44-Positive Feedback Loop." *Cell Death & Disease* 13 (2): 1–16. https://doi.org/10.1038/s41419-022-04607-0.
- Walton, Kristen L. W., Lisa Holt, and R. Balfour Sartor. 2009. "Lipopolysaccharide Activates Innate Immune Responses in Murine Intestinal Myofibroblasts through Multiple Signaling Pathways." *American Journal of Physiology. Gastrointestinal and Liver Physiology* 296 (3): G601-611. https://doi.org/10.1152/ajpgi.00022.2008.
- Wang, Dongli, Binlu Huang, Senyan Zhang, Xiaojuan Yu, Wei Wu, and Xinquan Wang. 2013. "Structural Basis for R-Spondin Recognition by LGR4/5/6 Receptors." *Genes & Development* 27 (12): 1339–44. https://doi.org/10.1101/gad.219360.113.
- "What Is Inflammatory Bowel Disease (IBD)? | IBD." 2022. April 27, 2022. https://www.cdc.gov/ibd/what-is-IBD.htm.
- Winkler, Juliane, Abisola Abisoye-Ogunniyan, Kevin J. Metcalf, and Zena Werb. 2020. "Concepts of Extracellular Matrix Remodelling in Tumour Progression and Metastasis." *Nature Communications* 11 (1): 5120. https://doi.org/10.1038/s41467-020-18794-x.
- Wipff, Pierre-Jean, Daniel B. Rifkin, Jean-Jacques Meister, and Boris Hinz. 2007. "Myofibroblast Contraction Activates Latent TGF-B1 from the Extracellular Matrix." *The Journal of Cell Biology* 179 (6): 1311–23. https://doi.org/10.1083/jcb.200704042.
- Wirtz, Stefan, Clemens Neufert, Benno Weigmann, and Markus F. Neurath. 2007. "Chemically Induced Mouse Models of Intestinal Inflammation." *Nature Protocols* 2 (3): 541–46. https://doi.org/10.1038/nprot.2007.41.
- Worthington, J. J., F. Reimann, and F. M. Gribble. 2018. "Enteroendocrine Cells-Sensory Sentinels of the Intestinal Environment and Orchestrators of Mucosal Immunity." *Mucosal Immunology* 11 (1): 3–20. https://doi.org/10.1038/mi.2017.73.
- Yamada, Kenneth M., and Michael Sixt. 2019. "Mechanisms of 3D Cell Migration." *Nature Reviews Molecular Cell Biology* 20 (12): 738–52. https://doi.org/10.1038/s41580-019-0172-9.

- Yamamoto-Furusho, Jesús K., Andrea Sarmiento-Aguilar, Joel J. Toledo-Mauriño, Katya E. Bozada-Gutiérrez, Francisco J. Bosques-Padilla, Manuel A. Martínez-Vázquez, Virgilio Marroquín-Jiménez, et al. 2019. "Incidence and Prevalence of Inflammatory Bowel Disease in Mexico from a Nationwide Cohort Study in a Period of 15 Years (2000–2017)." *Medicine* 98 (27): e16291. https://doi.org/10.1097/MD.0000000000016291.
- Zhang, Zhuobin, Akira Andoh, Osamu Inatomi, Shigeki Bamba, Atsushi Takayanagi, Nobuyoshi Shimizu, and Yoshihide Fujiyama. 2005. "Interleukin-17 and Lipopolysaccharides Synergistically Induce Cyclooxygenase-2 Expression in Human Intestinal Myofibroblasts." *Journal of Gastroenterology and Hepatology* 20 (4): 619–27. https://doi.org/10.1111/j.1440-1746.2004.03748.x.