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Review

Contribution of intestinal mucosa to the disease mechanisms of Spondyloarthritis: Unlocking new insights through bioengineered tissue models

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ABSTRACT

Spondyloarthritis (SpA) represents a spectrum of chronic inflammatory rheumatic disorders with similar articular and extra-articular features and a complex pathogenesis stemming from the interplay between genetics, environmental triggers, and immune system dysregulation. The significant prevalence of SpA and its onset in the working age population place a considerable burden on healthcare systems. Furthermore, many patients fail to achieve optimal treatment outcomes and present physical and psychological distress, underscoring the need for improved therapeutic approaches. The emerging concept of the gut-joint axis suggests that disturbances at the intestinal mucosal interface and microbiota level may initiate or amplify inflammatory responses, contributing to the clinical expression of SpA. Unfortunately, traditional pre-clinical in vitro and animal models have intrinsic limitations in replicating the intricate human intestinal mucosa anatomo-functional network, thus constraining translational research. Bioengineered tissue models (BTMs), including organoids, organs-on-a-chip (OoCs), and co-culture systems integrating human-derived cells, offer novel platforms that better mimic human tissue architecture and physiological responses. These advanced models hold promise in elucidating multifactorial disease mechanisms and expediting drug development, potentially facilitating personalized and more effective therapies. This article presents a narrative review of current knowledge about perturbations at the level of human intestinal mucosa during SpA and aims to critically synthesize the conceptual and experimental advances in the emerging field of BTMs to support future translational research in this field.

1. Introduction

Spondyloarthritis (SpA) is a family of chronic inflammatory rheumatic diseases best classified by the predominant pattern of involvement into axial (axSpA) and peripheral spondyloarthritis (pSpA). AxSpA includes both radiographic (formerly ankylosing spondylitis) and non-radiographic forms — based on the presence or not of structural damage to the sacroiliac joints on X-ray — while pSpA encompasses forms of psoriatic arthritis (PsA), reactive arthritis (ReA), and enteropathic arthritis (EnA) that mostly manifest with peripheral synovitis, enthesitis, and dactylitis.

Other than sharing several musculoskeletal clinical features, these conditions exhibit extra-articular manifestations, such as uveitis, skin and nail psoriasis, and gut inflammation to overt inflammatory bowel disease (IBD), underscoring their systemic nature [1–3].

SpA is the second most prevalent inflammatory rheumatic disease

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Table 1
Summary of evidence levels for key intestinal mucosa alterations in SpA.

Anatomo-functional element	Human SpA evidence	Human IBD / related immune-inflammatory diseases evidence	In vitro / ex vivo evidence	Animal evidence	Overall interpretation used in this review
Intestinal microbiota and dysbiosis	Moderate: multiple cross-sectional studies support altered microbial composition and links with disease phenotype/ activity, but temporality remains unresolved [22,28–34]	Supportive/indirect: related human intestinal inflammatory literature provides contextual support for dysbiosis as a disease-modifying factor [35]	Limited: current systems mainly model downstream consequences of dysbiosis rather than its emergence	Strong: experimental SpA models support a causal contribution of microbiota to disease expression [36–39]	In humans, dysbiosis is best interpreted as a robust association with biologic plausibility, whereas causality remains better supported in animal models than in patient studies.
Crosstalk between the intestinal epithelium and gut microbiota	Limited: in SpA, evidence mainly concerns receptor-related pathways and inflammasome-associated findings, while direct evidence in IECs remains scarce [40–47]	Moderate: human IBD literature supports epithelial TLR/NLR dysregulation and inflammatory receptor signaling [48,49]	Moderate: organoid and epithelial systems support mechanistic study of TLR- and inflammasome-dependent host–microbe interactions [50–52]	NA	The relevance of epithelial–microbial crosstalk to SpA is biologically plausible and partially supported, but much of the mechanistic framework is extrapolated from non-SpA human and in vitro studies.
Metabolite-mediated interactions between host and gut microbiota	Limited to moderate: altered tryptophan/indole balance and selected SCFA changes have been reported in AS/axSpA cohorts, but patient-level data remain sparse [53–59]	Supportive/indirect: broader human IMID literature supports metabolite-mediated regulation of mucosal and immune homeostasis [53,54,58]	Moderate: in vitro and BTM-oriented studies support functional effects on epithelial responses, bacterial growth, and barrier-related pathways [60,61,59,62–64]	Moderate: animal studies support immunomodulatory and arthritis-relevant effects of microbial metabolites [57]	In SpA, microbial metabolite alterations should currently be viewed primarily as human associations with mechanistic plausibility, rather than disease-specific causal pathways.
Intestinal barrier modifications	Moderate: in SpA, barrier-related alterations are supported by biopsy-based and histologic observations, including epithelial and gut-vascular junctional changes, permeability-associated markers, goblet-cell/mucin alterations, and Paneth-cell-related antimicrobial abnormalities [35,65,66,67,68]	Strong: IBD and related human intestinal inflammatory diseases provide the main framework for understanding barrier organization and dysfunction, including tight-junction remodeling, mucus impairment, and antimicrobial peptide dysregulation [65–67,69–76]	Moderate to strong: epithelial monolayers, organoids, and gut-on-a-chip systems enable functional study of permeability, junctional organization, mucus properties, and antimicrobial epithelial programs [77,78,79–94,95]	NA	Intestinal barrier dysfunction is one of the most relevant and biologically coherent domains in SpA, with direct human support at the descriptive level, but much of its functional and mechanistic interpretation still relies on IBD literature and experimental model systems.
Intestinal motility and its connection with the intestinal microbiota	Limited: in SpA, evidence is currently indirect and based mainly on increased prevalence of IBS/IBS-like symptoms rather than direct motility measurements [96,97]	Moderate: IBD literature supports dysmotility and ENS remodeling in intestinal inflammation [98,99,100]	Moderate: gut-on-a-chip and ENS-containing organoid systems provide functional platforms to test peristalsis-, neuroepithelial-, and microbiota-related effects [101,102–108]	Limited to moderate: animal studies support links between inflammation, adaptive immunity, and motility changes [109]	For SpA, the motility axis should currently be regarded as a testable and biologically plausible interface, not as a well-established disease mechanism.
Gut mucosa immune system and immune cells priming and recirculation	Moderate: human SpA data support mucosal type-3 immunity and altered IL-23/IL-22 signaling, but direct evidence on priming/recirculation mechanisms remains limited [110–119]	Strong: human IBD literature provides extensive support for Th17-rich mucosal inflammation and epithelial–immune crosstalk [116–118]	Moderate: immuno-organoids and immune-competent gut microphysiological systems provide functional support for modeling epithelial–immune interaction modules [120–125]	NA	Immune priming at the gut mucosa is central to the SpA hypothesis, but the specific sequence linking local activation to systemic recirculation remains incompletely demonstrated in human SpA.

NA Not addressed in the present Review.

after rheumatoid arthritis [4], typically affecting individuals in their early adulthood and leading to long-term functional impairment and major socioeconomic burden [5–9].

The dependence of diagnosis on the clinical evaluation of nonspecific symptoms with insidious onset (e.g., low-back pain), in the absence of reliable and specific biomolecular markers, accounts for the diagnostic delay, averaging 5 to 10 years in axSpA [10].

Despite therapeutic advances, including the introduction of therapies against key molecular targets such as tumor necrosis factor (TNF)-α, interleukin (IL)-17, IL-23, and janus kinase (JAK), primary non-response and loss of efficacy over time are frequent [11,12]. Furthermore, the absence of predictive biomarkers and reliable stratification tools hampers personalized treatment strategies, and clinical decisions — including allocation to targeted therapies — remain largely empirical.

The disease's origin is rooted in a complex interplay between genetics, with the human leukocyte antigen (HLA)-B27 allele of the major histocompatibility complex (MHC)-class I molecule-coding gene being a distinctive feature, and environmental factors such as infections and mechanical stress [13–15]. Immune-mediated responses involving both innate and adaptive mechanisms are characteristic of SpA, underpinning its clinical manifestations [16,17].

The multifaceted pathogenesis of SpA remains far from being fully understood. As recently highlighted, uncovering the deep complexity of axial SpA requires framing the disease well beyond classical inflammation to include bioenvironmental contributors like gut involvement and local tissue responses [18].

The unmet clinical needs associated with the disease, including diagnostic delay, therapeutic failure, and the lack of individualized approaches, underscore the necessity for novel, human-relevant models to improve our understanding of disease mechanisms and to guide

precision medicine.

A promising avenue in the quest to better understand SpA genesis is the exploration of the gut-joint axis. This model postulates a functional connection between the gut mucosa and joint compartment mediated by the immune system [19].

A growing body of evidence supports this model, highlighting both microbial triggers and intestinal barrier dysfunction ("leaky gut") as contributors to SpA onset, along with the high prevalence of clinical and subclinical intestinal inflammation in these patients [20,21], frequently overlapping with features of IBD — which co-occurs in up to 10% of cases — and sharing pathophysiological traits with SpA [22–24]. In particular, it has been suggested that modifications in the gut microbiota composition may influence the immune responses that trigger inflammation in patients.

As the gut-joint axis emerges as a central focus in translational SpA research, the intestinal mucosa takes on a critical role as a complex anatomo-functional interface that combines different players of interest in the pathogenesis of the disease. However, it becomes increasingly clear that accurately reproducing SpA intestinal mucosa-associated features — from host-microbiota interactions at the mucosal barrier to downstream immuno-inflammatory activation — presents unprecedented challenges due to the inherent limitations of traditional preclinical models.

Newly developed bioengineered tissue models (BTMs), namely organoids, organs-on-a-chip (OoCs), and multiple co-culture systems integrating human-derived cells, promise exceptional support in this regard thanks to their superior physio-anatomical accuracy, patient relevance, and mechanistic informativeness [25–27], but their application in SpA translational research has been largely neglected until now.

This article presents a narrative review of current knowledge about changes at the level of human intestinal mucosa during SpA and aims to critically synthesize the conceptual and experimental advances in the growing field of BTMs to support future translational research in SpA.

2. Anatomo-functional elements of intestinal mucosa relevant to SpA

In the following sections, the conceptual framework of intestinal involvement in SpA is discussed by integrating evidence from direct human SpA studies, related human immune-mediated intestinal diseases, selected in vitro systems, and, only where functionally informative to the narrative and not yet replaceable by human data, animal models. Accordingly, unless otherwise specified, the relevance of the processes described to SpA should be interpreted in light of this hierarchy of evidence rather than assumed to reflect equivalent disease-specific validation [TABLE 1].

2.1. Intestinal microbiota and dysbiosis

It has been widely demonstrated that the gut microbiota on the luminal side of intestinal mucosa is a determinant of gut homeostasis and a player in several diseases [23,126].

While it is now well established that intestinal bacterial colonization is essential in triggering the inflammatory response in animal models of inflammatory SpA, including the HLA-B27-positive rat [36,37] and the SKG mouse [38,39], the same contribution in human pathology is currently only hypothetical.

At the present stage, both 16S ribosomal RNA gene and shotgun sequencing studies showed that the gut microbiota of SpA patients has a peculiar composition compared with similar healthy controls and that the microbial signature varies in relation to the clinical and genetic phenotype of the disease [28–31]. The overrepresentation of some taxa across multiple SpA cohorts suggests a dysbiosis characterized by the emergence of specific strains [22]. For instance, in a recent study by Berland et al., a Bacteroides genus-dominated enterotype was shown to be more prevalent in patients with high disease activity and associated with low-diversity microbiota characterized by decreased abundance of Clostridiales [32]. Moreover, *Ruminococcus gnavus* was confirmed to be one of the top differentiating species in SpA patients compared to healthy controls and correlated with disease activity in patients with more pronounced gut inflammation due to concomitant history of IBD [30]. However, there is currently no evidence to determine whether dysbiosis precedes disease manifestations or results from changes in the intraluminal and mucosal environment caused by inflammatory processes.

Growing evidence indicates that the genetic background associated with SpA, specifically the presence of the HLA-B27 allele, may influence microbiota composition. Asquith et al. firstly compared the contents of the microbiota from stool and biopsy samples from 6 intestinal sites of HLA-B27-positive versus negative healthy adults undergoing ileocolonoscopy, showing substantial and consistent differences at multiple habitats within the gut, including decreased *Blautia* among the HLA-B27-positive subjects and associations between HLA-B27 genotype and a distinct overall microbiota composition in the SpA group [33]. In the Berland study, including 11 sibling trios (each consisting of an HLA-B27-positive SpA patient matched with an HLA-B27 positive healthy control and an HLA-B27-negative healthy control), HLA-B27 genotype was associated with microbiota changes even in the healthy siblings of SpA patients. Strikingly, microbiota characteristics of SpA patients and HLA-B27-positive healthy controls showed a certain degree of overlap, which was absent in the comparison between SpA patients and HLA-B27-negative healthy controls [32]. These data provide a further possible interpretation of how HLA-B27 may determine the disease, based on the concept of the interaction of the genetic background with the microbiota. However, another study comparing microbiota of HLA-B27-positive versus HLA-B27-negative offspring of AS patients and that of the former group of healthy subjects versus HLA-B27-positive pediatric SpA patients was not able to strongly support the concept of an association between genetic status and specific microbiota signature [34], with the limitation of different ages between groups. Ultimately, there is currently no mechanistic evidence linking HLA-B27, dysbiosis, and SpA onset due to the cross-sectional nature of the studies.

Resolving this "chicken-and-egg" dilemma — whether dysbiosis acts as a primary etiologic trigger or develops as a secondary consequence of early mucosal inflammation — strictly requires future research employing prospective, longitudinal study designs in incident SpA cohorts or high-risk individuals.

2.2. Crosstalk between the intestinal epithelium and gut microbiota

Crosstalk between intestinal luminal microorganisms and intestinal epithelial cells (IECs) represents the first layer of microbiota-host interaction. Direct influence of bacterial structures on IECs via receptor mechanisms and modification of mucosal layer homeostasis via the intraluminal metabolic profile have been advocated. In contrast, IECs can modulate the intestinal microenvironment by producing hormones, mucins, and antimicrobial peptides (AMPs) [127].

The receptor-like interaction between IECs and microbiota is mediated by several receptors with pattern recognition function for bacteria and microbes, among which the best characterized are Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [128], and the intracellular machinery mediating the connection between receptors and biological effectors, including the inflammasome [129]. Engagement of these receptors under conditions of altered mucosal homeostasis (as in infection or autoimmunity) is responsible for triggering a proinflammatory condition [130].

A possible role of TLRs in the pathogenesis of SpA has been hypothesized since the acquisition of evidence that certain Gram-negative bacteria can act as a trigger for ReA, a form of infectious disease-related arthropathy with a clinical phenotype falling within the spectrum of SpA. To date, there is some evidence regarding the relationship between polymorphisms in TLR2 and TLR9 and increased susceptibility to

developing SpA, independent of the presence of HLA-B27, and the demonstration of upregulation of TLR2, TLR4, and TLR5 in antigen-presenting cells in PsA and AS patients [40]. For that matter, the possibility of systemically modulating TLR-4 to mitigate joint inflammation is being scrutinized [41,42]. Still, evidence on the role of these receptors in IECs in SpA is scarce.

Narrowly correlated pattern recognition systems of the NLRs family and the inflammasome have also been the subject of interest in AS. A generic upregulation of the components of these signaling pathways, in particular NLRP3, has been reported during PsA and AS, noting a correlation with the activation levels of the IL-17 and IL-23 pathways [43], while the significance of NOD2- and NLRP3-related genetic variants on AS susceptibility is still ambiguous [44–46].

Recently, the activation pattern of the inflammasome was studied in AS patients in relation to intestinal dysbiosis [47]. Several inflammasome components, including NLRP3, NLRC4, and AIM2, were found to be upregulated in the inflamed gut of AS patients, correlating with the amount of adherent bacteria previously associated with AS dysbiosis. Interestingly, inflammasome inhibition could abolish IL-23 production otherwise induced by LPS exposure of human AS-derived monocytes.

2.3. Metabolite-mediated interactions between host and gut microbiota

The production and modulation of metabolites play a key role in mutualism between the host and gut microbiota. Strikingly, it has been estimated that 10% of all human metabolites have a microbial derivation [60]. At the same time, microbiota-induced effects on the immune system can be mediated by bioactive metabolites in addition to direct host-microorganisms interactions [61]. Thus, the study of the metabolome represents a pivotal aspect in the ecological characterization of a microbiota in specific conditions.

Exploration of the metabolome at the level of the different compartments affected by SpA is a field that has recently aroused interest [131,132]. However, the intestinal metabolome has been marginally explored during SpA.

In general, metabolomic analysis in humans of fecal samples or intestinal biopsies by various methods has been able to identify a differential metabolic profile between patients with SpA and healthy controls [53,54]. Of particular interest, because of the role of tryptophan in orchestrating intestinal homeostasis and modulation of inflammation and pain, was the finding of an altered balance of this amino acid and increased expression of its derivatives on the indole pathway distinguishing axSpA and Crohn's disease (CD)-associated axSpA patients, compared with HCs and patients with CD, and correlating with functional signature of the microbiota [55].

Shao et al., who also described the differential expression of several metabolite classes between AS and HC patients, including tryptophan, showed that fecal metabolic profiling was able to discriminate AS and RA patients from healthy controls but not RA patients from those with AS [56]. These data suggest the presence of a gut metabolic profile closely linked to the development of inflammatory joint disease, as otherwise demonstrated in mouse models [57].

One class of compounds particularly studied in the context of microbiota-host crosstalk, due to their preponderance as a product of bacterial metabolism, is short-chain fatty acids (SFCAs), a group of carboxylic acids derived from the anaerobic fermentation of dietary fibers that classically includes acetate, propionate, and butyrate. The potential role of SCFAs in immune-mediated diseases is interesting due to their ability to interact at various levels with cells of the immune system, and it is specifically the case in SpA, considering their ability to modulate the activity of T cells, including Treg cells and Th17 cells, promoting an anti-inflammatory balance [58]. At present, little has been studied in the intestines of patients with SpA, noting in one study a decrease in butyrate and propionate in fecal samples from patients with AS [56].

In addition, Lai et al. conducted a detailed characterization of the fecal microbiota and metabolome in a cohort of patients with AS [59], demonstrating a distinct metabolic profile compared with healthy controls and identifying some differentially expressed compounds, such as oxypurinol and biotin, capable of modulating in vitro the proliferation of bacterial strains relevant to the intestinal microenvironment of SpA, including *R. gnavus*.

2.4. Intestinal barrier modifications

The extent of molecules and microorganisms' translocation from the luminal compartment to the systemic circulation largely depends on the intestinal barrier function. In brief, mucosal integrity and permeability are the result of a nonlinear interaction between a chemical barrier, mainly carried out in the small intestine by AMPs produced by Paneth cells, and a physical barrier consisting of cell junctions between IECs and endothelial cells, glycocalyx on the microvilli of enterocytes, and the mucus layer provided by the goblet cells [35,65].

In the SpA gut-joint axis model, the intestinal barrier plays a dynamic role with an anatomo-functional impairment that allegedly varies with the progression and activity of inflammatory pathology [19].

2.4.1. Antimicrobial peptides

AMPs are a diverse group of molecules produced by specialized IECs called Paneth cells (PCs), as well as by immune cells in the gastrointestinal tract. These peptides are essential for sustaining host tolerance to the gut microbiota and innate immune defenses against intestinal pathogens [69].

The production and release of AMPs are tightly modulated in response to microbial presence, predominantly through Pattern Recognition Receptors (PRRs) activation. Defensins and cathelicidins represent the principal AMP families produced in the gastrointestinal tract, although IECs also release larger peptide variants with similar antimicrobial efficacy. AMPs strengthen IECs' capability to prevent bacterial translocation, and increasing evidence indicates that defensins modulate mucosal immune responses by recruiting monocytes, T cells, and immature dendritic cells to sites of inflammation [70].

Alterations in PCs and AMP production have been widely reported in ongoing IBD inflammation [71]. Data on SpA are more limited. Notably, Ciccia et al. first demonstrated that PCs are a major source of IL-23 in AS patients' homeostatic and inflamed intestinal mucosa. The same group, through the analysis of ileal samples from patients and controls, demonstrated that overexpression of AMPs by Paneth cells (particularly alpha-defensin 5 and lysozyme) is a feature of the early stages of inflammation in the intestines of SpA and CD patients and that the reduction of defensins in CD is mediated by the loss of Paneth cells in severe intestinal inflammation with mucosal disruption [66].

2.4.2. Intestinal tight junctions

Tight junctions (TJs) are specialized protein complexes located at the apical end of IECs and between gut endothelial cells, meant to seal the space between adjacent cells, regulating paracellular permeability. These complexes include transmembrane proteins such as claudins, occludin, junctional adhesion molecules (JAMs), endothelial cell-selective adhesion molecules, and cytoplasmic zonula occludens (ZO) proteins.

TJs are critical in preventing harmful pathogens, toxins, and antigens from directly entering the bloodstream. Importantly, tight junctions are dynamic structures that cytokines can modulate during inflammation and by microbial metabolites [72]. Zonulin, a family of structurally and functionally related proteins, is a major regulator of TJs competency and is known to increase intestinal permeability, particularly in the context of intestinal dysbiosis [73].

Notably, a significant downregulation of TJs components, including claudin-1 and -4, occludin, and ZO-1 for the epithelial side and JAM-A for the gut vascular barrier, was observed in the gut of patients with AS and gut inflammation compared with controls. In the same patients,

Box 1
Evidence and open questions on permeability in SpA.

Evidence for altered intestinal permeability in SpA is biologically plausible, but it remains methodologically heterogeneous and should be discussed by separating at least three partially overlapping barrier compartments:

- **Epithelial tight junction barrier.** The strongest direct human evidence comes from biopsy-based studies in AS with histologic gut inflammation, in which altered expression/localization of epithelial junctional proteins was observed together with increased permeability-associated circulating markers [67]. However, earlier functional studies yielded partly discordant results depending on the assay used: ^{51}Cr-EDTA studies suggested increased small-intestinal permeability in AS, whereas a lactulose–mannitol study did not demonstrate a consistent difference from controls [133–135]. Accordingly, replication in contemporary, independently phenotyped axSpA and pSpA cohorts remains limited.

- **Gut-vascular barrier.** The gut-vascular barrier should not be equated with epithelial tight junctions. In AS, reduced JAM-A/VE-cadherin-related endothelial barrier features, increased PV1, and elevated LPS, LBP, and iFABP have been reported, together with in vitro evidence that recombinant zonulin can alter endothelial junctional proteins [67]. Nevertheless, this vascular compartment has not yet been systematically replicated across independent SpA cohorts, and it remains unclear to what extent vascular leakage is secondary to epithelial injury, microbial products, or mucosal inflammation rather than a distinct primary pathogenic event [138,139].
- **Mucus barrier.** Mucus should be considered a third barrier layer rather than a passive by-product of epithelial damage. In SpA, the currently available evidence is largely indirect and histologic, including goblet-cell hyperplasia and increased mucin production in inflamed mucosa [140]. Whether these changes are compensatory, maladaptive, or insufficient to prevent bacterial encroachment remains unresolved. Direct functional assessments — such as mucus thickness, penetrability, bilayer organization, mucin glycosylation, and microbial localization relative to the epithelial surface — have not yet been systematically studied in independent SpA patient cohorts [141,142].

Methodological caution and preferred readouts. Circulating zonulin should be interpreted cautiously because widely used commercial ELISAs may detect proteins other than pre-haptoglobin-2 itself [136,137]. Future SpA studies should therefore avoid relying on a single surrogate marker and instead combine orthogonal readouts tailored to the compartment of interest. For epithelial permeability, these may include lactulose–mannitol or multi-sugar urinary tests, ex vivo or BTM-based transepithelial electrical resistance and tracer-flux assays, and confocal laser endomicroscopy where feasible [143,144]. For bacterial translocation and epithelial injury, LPS/LBP, sCD14, and iFABP can provide complementary — but still indirect — information; notably, in a recent longitudinal cohort of radiographic axSpA, LBP and calprotectin, but not zonulin, tracked with disease activity and treatment response [145]. For the mucus compartment, future studies should incorporate direct functional readouts, including mucus thickness, penetrability, goblet-cell differentiation, and mucin composition."

zonulin appeared to be upregulated and correlated with markers of increased gut permeability in vivo, including levels of LPS. A series of in vitro experiments showed that bacteria isolated from AS patients were able to significantly upregulate zonulin in human IECs [67]. However, these findings should be interpreted cautiously, because the permeability literature in SpA remains methodologically heterogeneous: earlier studies using ^{51}Cr-EDTA suggested increased small-intestinal permeability [133,134], whereas a lactulose–mannitol study did not confirm a consistent difference from controls [135], and the analytical specificity of widely used commercial zonulin ELISAs is debated [136,137]. For this reason, the currently available evidence is better viewed as supportive of barrier perturbation than as definitive proof of a single zonulin-driven causal pathway [Box 1].

2.4.3. Mucus layer

The intestinal mucus layer, primarily composed of mucin glycoproteins like mucins, serves as a significant barrier, physically and chemically preventing luminal microbes from accessing the intestinal epithelium and maintaining immune homeostasis. Recent findings suggest that the major mucus protein, MUC2, possesses bioactive properties, influencing tolerogenic responses in IECs and dendritic cells [74].

However, the intestinal mucosal layer is a highly complex and finely regulated system, for which over 20 mucins and a plethora of other molecules with active roles have been described.

While the depletion and structural failure of the mucus barrier are well-documented hallmarks of IBD — making it a potential therapeutic target in that context [75,76] — primary human data regarding mucus biology in SpA remains comparatively limited and suggests a distinct phenotype. Unlike the classical mucin depletion often seen in severe IBD, primary histological analyses of the intestinal mucosa in patients with SpA have demonstrated that local inflammation is instead associated with goblet cell hyperplasia and increased mucin production [140]. This suggests an early adaptive response aimed at maintaining mucosal integrity under inflammatory conditions. However, it is hypothesized that persistent inflammation may eventually compromise this barrier, facilitating microbial translocation and systemic immune activation.SpA dysbiosis itself may adversely affect the mucus layer's integrity, as certain commensal bacteria are involved in maintaining and regulating mucin production. Notably, certain strains of *Ruminococcus gnavus,* a species whose abundance in the microbiota of patients with SpA has been shown to correlate positively with disease activity, possess unique intramolecular trans-sialidase-based mechanisms for mucin degradation, which may impact the integrity of the intestinal mucus barrier and influence inflammatory processes in the gut [30,68]. It is crucial to recognize that mucolytic capacity exhibits significant strain-level heterogeneity. Given the lack of temporal proof, it remains undetermined whether — during inflammatory intestinal disease — the expansion of such specific mucolytic strains actively initiates barrier disruption or if they merely flourish in a pre-existing, inflammation-altered mucosal niche.

2.5. Intestinal motility and its connection with the intestinal microbiota

Intestinal motility is a major prerequisite in human intestinal physiology and is enabled by a complex neuromuscular anatomical system. It is virtually altered in all clinically relevant gastrointestinal tract diseases. For example, motility disorders are highly prevalent in IBD patients and characterize the subset of patients who have persistent gastrointestinal symptoms despite quiescent disease [98]. The mechanisms underlying dysmotility in inflammatory disease remain unclear. Still, the possible direct contribution of the local proinflammatory milieu and the immune system in influencing the function of the enteric neuromuscular complex has been highlighted [99,109].

Structural alterations with depletion and remodeling of enteric neurons and enteric glial cells have been documented in IBD mediated by the response of nerve endings located in close correspondence to the mucosal surface of the absorptive epithelium to various chemical stimuli, including TLR signaling and 5-hydroxytryptamine and SCFAs exposure [146,100].

These observations connect to growing evidence supporting the bidirectional relationship between intestinal dysmotility and dysbiosis, with a tendency for reciprocal amplification between the two conditions [147,148].

Notably, a high prevalence of irritable bowel syndrome (IBS) and IBS-like symptoms has been described in patients with SpA [96,97]. Therefore, it can be speculated that a certain degree of dysmotility is present in SpA patients with intestinal inflammation. However, rigorous studies using quantifiable instrumental methods of dysmotility or structural involvement of the enteric neuromuscular complex in SpA are needed. Accordingly, the enteric neuromuscular axis should presently be regarded less as an established SpA mechanism than as a biologically plausible and testable interface linking inflammation, epithelial stress, microbial ecology, and gut symptoms.

2.6. Gut mucosa immune system and immune cells priming and recirculation

The immunopathogenesis of SpA is highly complex and seems to vary substantially with clinical phenotype. Virtually all components of both innate and adaptive immunity have been implicated in disease onset and maintenance, but most of the mechanisms linking immune activation and clinical manifestations due to inflammation in the target tissue are still poorly understood [16,17].

In brief, T cells appear to play a central role in both SpA, primarily due to their ability to integrate signals from both immune arms. Notably, the pathogenic contribution of type-3 immunity with Th17 cell-mediated humoral and cellular responses is supported by the clinical efficacy of anti-cytokine therapies targeting this axis, at least for articular and spinal involvement in SpA and skin psoriasis. Potential alteration of regulatory T cells has also been described [110,111].

Although B cell involvement and the associated humoral response are generally regarded as secondary in SpA, multiple studies have identified abnormalities in B cell compartments. However, the clinical significance of these findings remains unclear [112,113].

While a comprehensive review of immune mechanisms involved in the pathogenesis of SpA is beyond the scope of this work, the potential of BTMs in assessing immune system priming at the level of gut mucosa should be emphasized.

The gut-associated lymphoid tissue (GALT), including intraepithelial and lamina propria cells working in close contact with specialized epithelial microfold cells, represents a critical component of the mucosal immune system and is the primary site for the initiation and regulation of immune responses to antigens encountered in the GI tract. In this regard, GALT serves both as a sophisticated immunological network dedicated to maintaining intestinal homeostasis after continuous exposure to diverse dietary and microbial antigens, and as a fence defending the host against pathogenic microorganisms [114].

Few studies have assessed GALT components in SpA patients. In general, type-3 immunity, including Th17 cells as main effectors, is prevalent in the intestinal mucosa, where these cells play a key role in maintaining tissue homeostasis and defending against pathogens [115]. However, Th17 cells are also abundant in the inflamed gut tissue of patients with IBD, where their frequency and expression of Th17-associated cytokines have been positively correlated with disease activity, endoscopic and histological severity, and levels of inflammatory markers [116].

This duality of Th17 cells in the gut is thought to arise from distinct upstream signals. IL-23-independent IL-17 production by innate and innate-like lymphocytes promotes mucosal protection by enhancing epithelial barrier integrity, stimulating antimicrobial peptide secretion, and supporting mucus production by goblet cells. In contrast, IL-23-dependent IL-17 production by Th17 cells has been implicated in determining a pro-inflammatory status [117,118].

In the terminal ileum of patients with axSpA, elevated levels of IL-23 and IL-22, but not IL-17, have been observed, differing from the serum profile, where both IL-23 and IL-17 are raised. This suggests a tissue- and disease-specific regulation and function of the IL-17 axis [119].

Notably, dysbiosis-driven expansion of intestinal IL-17 and IL-22-producing innate lymphoid cells (ILCs), a distinct family of immune cells that lack antigen-specific receptors and lineage markers, has been described in SpA [149]. Regner et al. reported a reduced number of intraepithelial lymphocytes (IELs) in patients with axSpA compared with controls. Furthermore, IELs from patients with CD and axSpA produced increased levels of TNFα, but only IELs from patients with CD produced more IL-17 than controls [150]. An older study suggested that the ileal immune infiltrate in patients with IBD and AS is mainly sustained by αβ cells, rather than γδ cells, a T-cell subset with a homeostatic role, gaining interest for their capacity to produce significant amounts of IL-17 [151].

The growing body of published studies demonstrating the presence in circulation and localization in joint and periarticular tissues of cells closely associated with the gut mucosa, including mucosa-associated invariant T (MAIT) cells responsible for recognizing and responding to microbial metabolites at barrier surfaces, represents one of the most important pieces of evidence supporting a mechanistic link between the intestine and the joint, even if rigorously demonstrating recirculation of human immune cells from the gut to the joint is technically challenging [152,153].

3. Current cellular and animal SpA pre-clinical models and their limitations

Most pre-clinical and translational research in SpA relies on primary or immortalized human cell lines — mainly of hematopoietic or epithelial lineage — cultivated according to standard methods and animal disease models. These experimental models have been well characterized and tested over the years, allowing for the collection of valuable information to clarify some of the mechanisms underlying SpA [154,155]. In addition, these models are currently used in various stages of preclinical testing of candidate therapeutic molecules. However, they do present intrinsic limitations [156].

The use of patients' and controls-derived cells has been mainly aimed at characterizing the phenotype and activation patterns of immune cells of SpA. Classical workflows include collecting and isolating peripheral blood mononuclear cells or cells in specific niches, such as those associated with the intestinal mucosa, their stimulation, and subsequent phenotyping [157–159].

The main limitation of this approach is the reduced availability and viability of primary cells with traditional cell culture techniques and the low physio-anatomical representation of these in vitro settings. Furthermore, the need for large cell quantities often necessitates invasive sampling of hematopoietic tissues or solid organs, limiting access to relevant human material.

Immortalized cell lines, predominantly of monocyte-macrophage lineage and seldom of lymphoid derivation, have been used to validate the observations derived from primary cells. Their main advantage lies in their wide availability, allowing also genetic manipulation. For example, the induced expression of HLA-B27 has been used to verify this allele's pathogenetic contribution [160–164]. However, the relevance of this in vitro model in the study of disease and patient-specific mechanisms is controversial due to the lack of sophistication and strong biases related to the particular genetic background of these lines and their harnessing [165].

In general, current approaches largely neglect the use of human cells to model tissue and organ-level structures outside the immune compartment — despite their relevance for accurately representing the gut-joint axis.

Animal models are the preferred alternative to study disease pathogenic SpA processes in a complex in vivo setting and access all tissue compartments affected by the disease, including synovium, tendons, bone, intestine, hematopoietic system, and skin. Several animal models have been used over the years for SpA [155,166]. Those considered most relevant are rats transgenic for HLA-B27 and human beta-2-microglobulin, which spontaneously develop a phenotype mimicking the human disease [167]. Still, interspecies biological differences remain a major transversal limitation of animal models. At the same time, their cost and limited availability due to the need for dedicated facilities, and growing ethical concerns limit their use [168]. In the specific context of gut-joint axis modeling, the inability to precisely control the intestinal microbiota in laboratory animals hinders the implementation of reductionist strategies aimed at dissecting specific host-microbe interactions [169].

In brief, current in vitro cell cultures and animal models fall short of accurately representing the complex interactions in human pathophysiology implied in the SpA gut-joint axis. They either oversimplify the disease processes, overlook inter-individual variability, or fail to

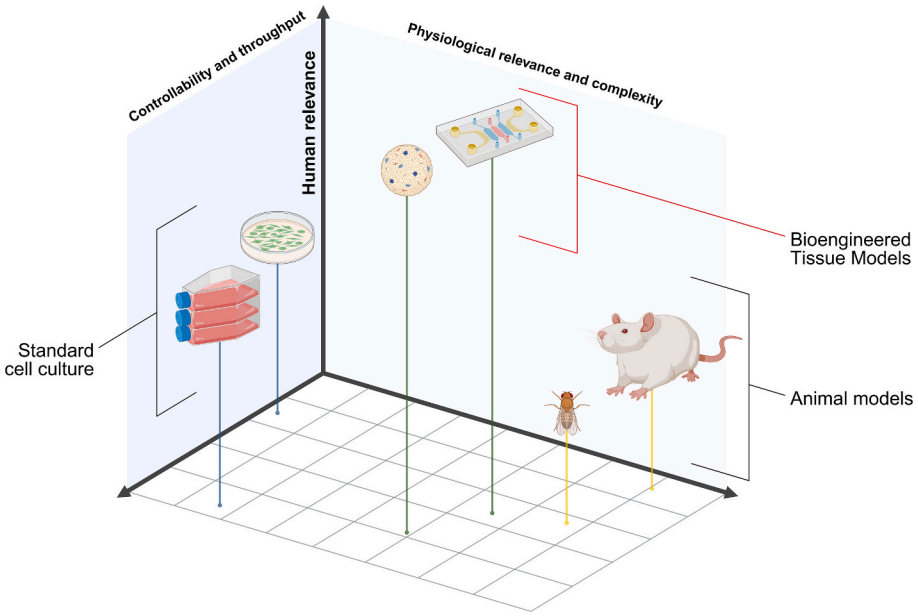

Fig. 1. Comparison of Bioengineered Tissue Models with other main types of preclinical models. Platforms such as organoids and organ-on-a-chip offer an ideal balance between physiological complexity and scalability compared to traditional models (i.e., standard cell cultures and animal models) but with greater relevance for translation into human medicine (patient-derived tissue). Created with BioRender.com

replicate the human gut and joint microenvironments. The nature of these models can limit the efficiency of the translational process from bench to bedside and pre-clinical testing, increasing the time and costs of research and drug development. [Fig. 1].

4. General characteristics and opportunities of bioengineered tissue models

Organoids are 3D structures grown from stem cells (SCs) meant to mimic the complexity of the tissues from which they are derived. OoCs, on the other hand, incorporate living cells into microfabricated devices that replicate key aspects of organ structure [25]. Both models offer unprecedented opportunities to emulate human physiology and disease processes more accurately. Importantly, they have the potential to provide complementary insights, with organoids excelling in mimicking tissue- and organ-level morphological organization, and OoCs enabling the study of organ interactions and responses to different stimuli under mechanical forces and chemical gradients.

One of the basic principles of BTMs is the exploitation of the potential of human SCs. BTMs of many tissues and organs, particularly those of the epithelial type, can be derived from stem cells found in specialized tissue niches, such as the epithelial crypts of the digestive tract. These cells, called adult stem cells (ASCs), have multipotency characteristics. Their main advantage is that they can give rise to organoids quickly and robustly under the right tissue-specific culture conditions [170,171]. They are ideal for generating patient-derived organoids (PDOs) with a stable karyotype [172,173]. Their proliferative nature makes them suitable for applying high-throughput workflows and possible genetic manipulation (i.e., gene disruption, knock-in, or controlled transgene expression) [174].

One of their main disadvantages is their reduced versatility in tissue generation, typically limited to the lineage of the original stem cell niche. Pluripotent stem cells can be equally used for the generation of BTMs. The classical source of pluripotent stem cells is embryonic stem cells. Human embryonic stem cells have been successfully used to generate different types of organoids [175–177]. However, blastocyst-derived cells have limited application because of their scarcity and ethical concerns. Therefore, induced pluripotent stem cells (PSCs) are commonly adopted. These cells can be generated by reprogramming somatic cells from a given individual, providing a highly versatile platform for modeling human biology [178,179]. Directed differentiation protocols enable the formation of complex organoids comprising multiple cell lineages, including those originating from different germ layers. Importantly, induced PSCs-derived organoids retain the donor's genetic background, although they may exhibit lower genomic stability than adult stem cell (ASC)-derived models. Despite their potential for disease modeling and personalized medicine, the increased technical complexity and cost of induced PSCs limit their use in standard experimental settings.

Another key element in the development of BTMs, necessary for achieving a higher level of morpho-functional sophistication, is the establishment of an extracellular environment that can provide adequate structural support and biochemical signaling. The basic approach is the cultivation of stem cells in an extracellular matrix surrogate [180]. Stem cells placed in this substrate tend to develop three-dimensional structures with reproducible polarization. When spatial regulation of cell proliferation is needed, advanced bioprinting techniques employing synthetic hydrogels can be used to engineer customizable extracellular matrix scaffolds [181].

Tissue-specific organoid growth relies on a tailored combination of biochemical signals, delivered via culture media enriched with selected growth factors and small-molecule modulators. By manipulating them, it is possible to control growth conditions, for example, by favoring a program of proliferation rather than tissue differentiation, to fit specific experimental needs [182,77].

Finally, the use of bioengineered “On-a-chip” systems allows the integration of cell cultures, structural support substrates, and culture media with micro-physiological modules, further expanding the flexibility and experimental potential of BTMs [183]. Common components include nano- or micro-porous membranes, microfluidic modules, and mechanical strain units. These systems enable multi-compartmental interactions between stem cells and niche factors - such as chemical gradients - and can replicate physical stimuli encountered in vivo, including peristalsis, compression, traction, stretching, and shear stress. In particular, microfluidic gut-on-a-chip systems that combine luminal flow with cyclic mechanical strain are necessary to induce a true three-dimensional epithelial architecture - complete with crypt-villus folding and polarized barrier function - critical for modeling host–microbiota

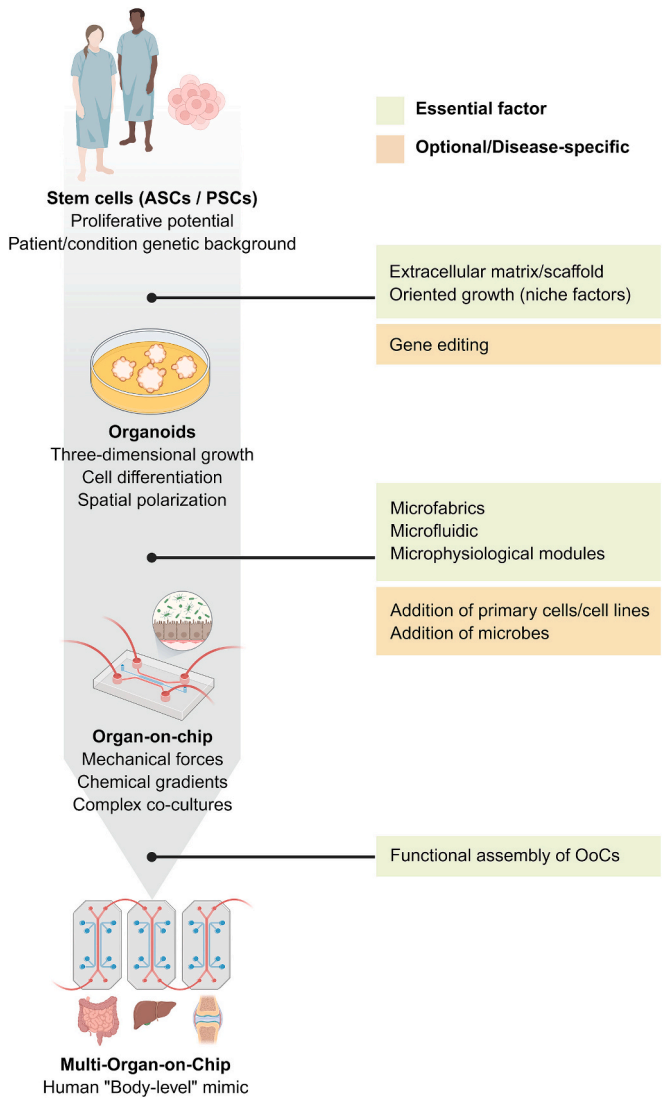

Fig. 2. Main features of BTMs. Different types of BTMs (illustrated in the figure by organoids and organ-on-a-chip devices) offer varying levels of sophistication, including different biotechnological features. ASC Adult stem cell; PSC Pluripotent stem cell; OoC Organ-on-a-chip. Created with BioRender.com

interactions [101,184]. In addition, OoCs allow, via microfluidic channels, the connection of different chips, allowing the study of remote effects of controlled variations within a chip (multi-organ-on-a-chip). [Fig. 2].

For pragmatic SpA-oriented applications, several technical points deserve explicit consideration. PDOs should preferably be established from endoscopic biopsies or surgical specimens, expanded under standardized stem-cell conditions, cryobanked, and used within a predefined passage window, because passage history can materially influence differentiation state and assay variability [185–187]. Epithelial fidelity should then be verified by structured quality-control workflows, ideally integrating single-cell RNA sequencing benchmarked against reference intestinal atlases or, more pragmatically, orthogonal marker panels covering stem/progenitor cells (e.g., LGR5, OLFM4), absorptive enterocytes (ALPI/VIL1), goblet cells (MUC2), enteroendocrine cells (CHGA), Paneth/secretory lineages (LYZ/DEFA5), and junctional/polarity markers such as ZO-1 and E-cadherin [188,189]. When organoids are adapted to 2D monolayers or microfluidic chips, apical-basolateral polarity should be demonstrated rather than assumed, using structural and functional readouts such as ZO-1 localization, brush-border organization, transepithelial electrical resistance, permeability assays, or differential apical/basolateral transport [190,191]. Likewise, for microbiota-facing platforms — especially those incorporating strict anaerobes — oxygen gradients should be directly measured with integrated sensors or equivalent validation approaches, because the ability to maintain a low-oxygen luminal compartment is a key determinant of both microbial viability and epithelial behavior [192]. Finally, future SpA studies should explicitly account for batch effects and inter-donor variability by harmonizing matrix/media lots and passage number, randomizing experiments across runs, incorporating technical replicates within donor and independent biological replication across donors, and prespecifying donor numbers according to the primary endpoint and expected between-donor variance [186,187].

5. Preclinical modeling of intestinal mucosa through bioengineered tissue models

Despite the growing technological maturity of BTMs, their application remains conspicuously underexplored in SpA, with no disease-specific validated organoids and organ-on-a-chip platforms for intestinal modeling. Furthermore, as highlighted in the prebvious evidence-mapping section, human SpA intestinal data remain conspicuously observational in several critical areas (e.g. the mucosal metabolome and the intestinal motility). In contrast, looking at the closely related field of IBDs, intestinal organoids and microfluidic gut-on-a-chip systems have seen thorough development and characterization in several research settings. This robust preclinical groundwork underscores a critical opportunity — indeed, a need — to extend and adapt these sophisticated models to unravel the intestinal mucosa contributions to SpA pathogenesis, starting from the relevant anatomo-functional elements.

At present, direct intestinal BTM data in bona fide SpA contexts remain limited to a small number of proof-of-concept studies, most notably the SpA microbiota triple co-culture model [78]. By contrast, most organoid, gut-on-chip, and immune-co-culture studies discussed below were developed in IBD, infection biology, cancer research, or general mucosal immunology and are cited here primarily as enabling methodological frameworks that can be adapted to SpA-oriented questions, rather than as already validated SpA models. Accordingly, unless explicitly stated as SpA-derived or SpA-specific, the following examples should be interpreted as translationally informative and hypothesis-generating, but still inferential with respect to SpA.

5.1. *Intestinal microbiota and dysbiosis*

The first common approach to translating patient-derived gut microbiota within a preclinical model can be integrative. In this case, the entire bacterial consortium from a specific biological niche is used, in an effort to preserve its complexity and composition [193,194]. Beterams et al. first described a method for placing a fecal isolate obtained from patients with SpA or healthy controls into a multiple immune-epithelial cell culture system. Using this setup, a differential epithelial response was shown in response to the microbiota of a patient dominated by *Prevotella spp*, while not specifically addressing the problem of maintaining an anaerobic environment [78]. Through the use of a refined gut-on-a-chip system, Jalili-Firoozinezhad et al. provided the proof-of-concept that patient-derived gut microbiota can be maintained in a controlled anaerobic microenvironment, characterized by a reproducible oxygen gradient, in order to best represent the physiological conditions of interaction between microbiota and intestinal mucosa [195]. Importantly, the epithelial cells used in this primary gut-on-a-chip model were isolated directly from patients using resected intestinal tissue as the source and expanded through organoid culture, thus providing an important framework for the use of patient-derived cells, which is of central importance in modeling a disease with a strong genetic background with potential effect on the microbiota, such as SpA. An alternative method of co-culturing IECs and human microbiota obtained by processing a fecal sample was also described by Shin et al.,

demonstrating the ability of bacteria isolated from feces to recolonize the epithelium with a clearly identifiable morphological organization in a microfluidic system equipped with an anoxic compartment [196].

This inclusive approach is particularly appealing for studying the global effect of a well-characterized microbiota but may render it difficult to isolate the biological effect of a distinct strain. Thus, in the specific context of SpA, it is worth considering also a reductionist approach for modeling the gut microbiota, implementing bacterial consortia composed of the strains deemed of interest, or studying specific individual strains selected by culturomics techniques [197–199]. Some of these strains may be particularly sensitive to oxygen, making them particularly difficult to study under normal culture conditions.

This reductionist approach becomes particularly useful when using source material other than feces (e.g., intestinal biopsies), to select the mucosa-associated microbiota. Indeed, the luminal and mucosa-associated microbiota have been shown to have well-separated compositions, especially for proximal luminal sites [200–202]. Moreover, the mucosa-associated microbiota may play a particularly important role in SpA, since in the gut of ankylosing spondylitis (AS) patients, the proportion of adherent and invasive bacteria correlates with the histologic degree of inflammation [67].

It should be emphasized that, when translating these microbial signatures into functional inputs for BTMs, researchers must critically address sampling biases. The composition of the luminal microbiota, typically assessed via easily accessible fecal samples, diverges significantly from the mucosa-associated microbiota that directly adheres to the epithelium. Because the most critical immune-priming and barrier-disrupting events in SpA likely occur directly at this epithelial interface, prioritizing mucosa-associated microbial consortia (obtained via intestinal biopsies) over fecal isolates could be paramount for accurately capturing the localized, disease-relevant crosstalk in bioengineered in vitro platforms.

Shah et al. demonstrated, in their gastrointestinal human–microbe interface microfluidic system, how a gut-on-a-chip type model is capable of capturing different transcriptional, metabolic, and immunological responses following co-culture with different isolated commensal strains [203]. Furthermore, Zhang et al. demonstrated through a microphysiological system how it is possible to successfully establish co-culture over an extended period (spanning weeks) of super oxygen-sensitive bacteria such as *Faecalibacterium prausnitzii* [204]. This is particularly interesting, as one of the top differentiating species confirmed to be most highly expressed in SpA is *R. gnavus*, a bacterium belonging to the phylum Firmicutes that cannot unfold at atmospheric amounts of oxygen [205].

Remarkably, the ability to maintain a more or less stringent anaerobic condition segregated to the apical compartment of an intestinal epithelium co-culture model represents one of the major technical advantages achieved by microfluidic OoCs systems in modeling microbiota-host interaction.

5.2. Crosstalk between the intestinal epithelium and microbiota

Epithelial organoids are known to be competent in TLR-related expression and signaling [206]. In addition, they are effective in representing the expression profile of TLRs compared across different gastro-enteric tract localizations [207]. As demonstrated by Neal et al. using intestinal organoid models, TLR4 appears to play an important role in determining the rate of proliferation and apoptosis in the stem cells in crypts in a p53-dependent manner [208]. Investigating these aspects in a patient-derived model for SpA, may be of particular interest, considering that IECs-associated TLRs seem to have a specific activation profile during IBDs [48,49] and that the stem cell niche seems extremely susceptible to pro-inflammatory changes determined by immune cells [209] or metabolic conditions [210]. Not surprisingly, TLR-4 targeting has been hypothesized as a strategy to alleviate the manifestations of gut inflammation [211].

Samuel et al. specifically examined TLR expression in organoids derived from human colon, finding a level of transcript for all TLRs 1 to 6 comparable to that of native tissue. Strikingly, they reported a large difference in the functionality of each of the TLR forms, finding a significant biological response, in terms of pro-inflammatory cytokine production, only after TLR-5 challenge with flagellin, but not after challenge with a selective TLR-2 and 4 agonist [50].

Inflammasome-induced cell death triggered by invasive bacteria is an innate response meant to restrict bacterial loads within the epithelium, confine barrier disruption, and prevent further detrimental inflammatory tissue damage. Pathogen restriction is mediated via the specific extrusion of dying IECs and membrane permeabilization. These mechanisms can be studied in real-time in intestinal epithelial BTMs with a high temporal and spatial resolution [51].

Using human intestinal organoids and organoid-derived epithelial monolayers, Ventayol et al. demonstrated how the epithelial response to invasion by flagellated bacteria is characterized by a rapid protective contraction of the epithelium coordinated by the NAIP/NLRC4 inflammasome in the sublithic phase [52]. Interestingly, this kind of response occurs in the absence of other mucosal cell types and relies directly on epithelial actomyosin contractions but may result in early destruction of barrier integrity in the case of a functional Caspase-1 or Gasdermin D deficiency. Since disruption of the epithelial barrier is considered a feature of the inflamed mucosa in SpA and IBD, BTMs could be used to study this aspect in relation to inflammasome activation in these conditions.

Crucially, it is important to distinguish the specialized role of this epithelial NAIP/NLRC4 inflammasome — which primarily orchestrates localized pathogen restriction and cell extrusion — from the activity of myeloid-derived inflammasomes (such as NLRP3 in lamina propria macrophages), which predominantly drive classic pro-inflammatory cytokine cascades (e.g., IL-1β, IL-18) and subsequent immune cell recruitment [212,213]. Advanced BTMs, particularly dual-channel microfluidic organ-on-a-chip platforms, offer the unique capability to compartmentalize these distinct responses by physically separating the epithelial barrier from the underlying immune compartment, thereby enabling precise, cell-type-specific dissection of inflammasome signaling triggers and kinetics [214].

5.3. Metabolite-mediated interactions between host and gut microbiota

In consideration of their inherent characteristics, BTMs are flexible and realistic models that allow the expansion of the characterization of metabolic processes at the level of the intestinal mucosa. Beyond the translation of mass spectrometry-based metabolomics for in-depth mapping of the biological effect of various compounds and molecules on an advanced human in vitro cell model [215,216], the morpho-structural sophistication of organoids makes it possible to apply advanced imaging methods for bioenergetic characterization of cell function with spatial resolution [217].

Both the metabolic control of the intestinal stem cell compartment and the interaction between metabolites, microbiota, and host are aspects of interest in the field of intestinal inflammation in SpA and IBD, but they are largely unexplored. The use of BTMs has important potential in this respect. For example, Rodriguez-Colman et al. utilized small intestinal organoids to demonstrate metabolic compartmentalization within the intestinal epithelium, finding that Lgr5+ stem cells exhibit high mitochondrial activity. In contrast, neighboring Paneth cells show increased glycolytic metabolism. Their research revealed a lactate-based metabolic symbiosis between these cell types, with Paneth cells producing lactate as a respiratory substrate to support oxidative metabolism in stem cells [218].

Additionally, the role of mitochondrial pyruvate import in the stem cell compartment was confirmed in human intestinal organoids through a pharmacological inhibition approach [219]. Schilderink et al. showed that the butyrate-induced enzymatic profile in human intestinal

organoids contributed to the maintenance of gut homeostasis through the modulation of retinoic acid production [220], while Bellono et al. were able to use intestinal organoids to demonstrate the role of serotoninergic enterochromaffin cells as chemosensors in the gut epithelium to detect and transduce environmental, metabolic, and homeostatic signals from the intestinal mucosa to the nervous system [221].

The use of gut-on-a-chip type platforms has further expanded the modeling possibilities, considering that the use of microfluidics for physiological-mimicking nutritional supplementation can have a major impact on cellular metabolism and function. Donkers et al. used an innovative microfluidic platform based on intestinal on-a-chip implantation to provide proof-of-concept of the dynamic interaction between prebiotics, bacteria, and indices of barrier function and epithelial inflammation [62]. At the same time, another study carried out on an integrated colon-on-a-chip platform (Emulate) showed, with a multiomics approach, how the susceptibility of human intestinal epithelium to pathogens such as enterohemorrhagic *Escherichia coli* is enabled by metabolic factors specific to the human intestine [63].

Notably, organoids have recently been validated as a human-derived model for the transport and metabolism of nutrients and drugs [64].

Because patient-level evidence in SpA remains limited and is currently based mainly on metabolomic association studies, the immediate value of BTMs in this field will be to test whether the altered tryptophan/indole balance reported in intestinal tissue from axSpA and CD-associated axSpA [55] and the reduced fecal levels of selected SCFAs described in AS [56], within the broader context of microbial metabolite dysregulation in SpA [53,54,58], are functionally active at the epithelial level rather than merely associative. In practical terms, defined SCFAs or indole-pathway metabolites should be tested in patient-derived organoids, organoid-derived monolayers, and, when host–microbiota co-metabolism is central to the hypothesis, gut-on-chip systems [217,63,64]. For barrier-centered hypotheses, a supportive effect should be defined prospectively by increased transepithelial electrical resistance, reduced FITC-dextran or analogous tracer flux, restoration of junctional organization, and attenuation of epithelial cytokine release after inflammatory or microbial challenge [62,79,84,88]. For cell-state and lineage-centered hypotheses, the same perturbations should be assessed for their ability to shift epithelial programs toward less inflammatory and more homeostatic states, including indole-responsive epithelial signaling, to preserve mitochondrial fitness in the LGR5+ crypt compartment, and to maintain Paneth-cell antimicrobial programs under challenge conditions [217–219,81–83]. In this setting, a convincing mechanistic effect should be considered one that is reproducible across independent donor-derived lines and concordant across at least one functional endpoint and one molecular endpoint, rather than inferred from metabolite exposure alone. Importantly, these effects should not be assumed to be uniformly protective: acetate has shown barrier-protective and anti-inflammatory effects in patient-derived epithelial monolayers, whereas butyrate responses can diverge according to inflammatory context and epithelial state, underscoring the need for dose titration, donor stratification, and matched healthy-control benchmarks [222–224].

5.4. Intestinal barrier modifications

Modeling the lumen-capillary tissue interface requires the incorporation of the core chemical and physical elements of the intestinal epithelial barrier to replicate an organ-level, tissue-specific milieu of the human live gut. BTMs are ideal systems for integrating and dynamically manipulating them to dissect the contribution of individual elements to the pathophysiological processes of interest, such as intestinal mucosa inflammation and crosstalk with the immune system [79].

A comprehensive approach has been proposed for modeling the intestinal barrier by integrating BTMs with a high level of sophistication that can implement assays of permeability, morphology, and integrity, including transepithelial electrical resistance [80].

However, it is also worth considering the specific determinants of barrier function of interest in SpA and how BTMs are leveraged to explore them.

5.4.1. Antimicrobial peptides

The refinement of intestinal organoid culture conditions has enabled preclinical models of patient-derived epithelium that can effectively represent even specialized subtypes of IECs, including Paneth cells, Tuft cells, and enteroendocrine cells [77]. This possibility makes intestinal organoids a valuable preclinical model for studying the role of AMPs. Notably, the use of these models has provided developmental insights into Paneth cells, including their close dependence on IL-22 signaling [81] and the close dependence of their density on intestinal crypt morphology [82], while the introduction of anatomical sophistication through a villi-crypts biomimetic surface has demonstrated to enhance antimicrobial capacities of patient-derived epithelium against pathogens [83].

5.4.2. Intestinal tight junctions

Research using human intestinal organoids offers the potential to gain insights into the regulation of intestinal permeability and the stimuli that can increase it. Compared to traditional epithelial models based (i.e., Caco2 monolayers), human intestinal organoids have shown greater granularity in their ability to demonstrate the hierarchical sequence of changes leading to the loss of barrier function, including the redistribution of junctional proteins from the cell border [84]. Different studies have extensively demonstrated that exposure of intestinal organoids to pro-inflammatory cytokines — including IL-1β, IL-6, and TNF-α — significantly decreased the expression of tight junction proteins such as ZO-1 and occludin. Clinically used treatments such as glucocorticoids and anti-TNF-α agents partially restored barrier function, although not all parameters were normalized [85–87].

Notably, Nwako et al. suggested that modification of tight junctions is not the sole determinant of inflammatory impairment of the intestinal barrier. Using monolayers derived from human intestinal enteroids engineered to lack enteroendocrine cells (EECs), they first showed that EECs are essential for maintaining barrier integrity in the differentiated intestinal epithelium. They then identified two EEC-derived hormones — peptide YY peptide tyrosine-tyrosine and somatostatin — that restore barrier function following pro-inflammatory stimulation with TNF-α. Importantly, this hormone-induced rescue of barrier function arose not from increased expression of junctional proteins (ZO-1, occludin, claudin-2) but from qualitative changes in their epithelial localization, highlighting the underexplored role of EECs in maintaining barrier integrity [88].

5.4.3. Mucus layer

Intestinal organoids, derived from human pluripotent stem cells or adult intestinal stem cells, can be cultured to form a functional mucus layer, facilitating studies on mucus dynamics and barrier function [89]. However, traditional organoid cultures often encapsulate mucus within their luminal space, limiting accessibility for experimental manipulation and requiring labor-intensive micro-injection techniques.

Notably, Beterams et al. described a triple co-culture system (bacteria, and immortalized mucus-producing epithelial and monocyte lines) with static Transwell-based BTMs to test the effect on the intestinal mucosa of microbiota derived from patients with SpA [78]. In this system, the effect on the mucosal layer was measured as its thickness by immunofluorescence, highlighting a reduced thickness and greater variability of the mucosal layer by the epithelium exposed to the fecal slurries of SpA patients compared to healthy controls. However, the authors could not assess the effect on epithelial cells derived from the patient or the contribution of dynamic physiological elements such as peristalsis and flow.

To overcome previous limitations, integrating patient-derived with microfluidic OoC systems has been shown to enhance the physiological

relevance of in vitro models of the human intestinal barrier [90,91]. A refined human colon-on-a-chip device has been developed that supports the spontaneous differentiation of goblet cells and the formation of a mucus bilayer with structural and functional properties akin to the native human colon. This platform enables real-time analysis of mucus accumulation and responses to inflammatory stimuli, providing insights into barrier integrity under various conditions [92]. A similar system has been proposed to assess mucus secretion in the small intestine under physiological conditions [93], but could be potentially adapted to specific modeling of inflammatory conditions.

Finally, a fluidic system has been developed to accurately study the diffusion dynamics of bacterial compounds (specifically endotoxins) through the intestinal mucosal layer [94]. The implementation of such a system is of major interest for studying the pathogenicity properties of individual bacterial species of interest in SpA or complex microbiota obtained directly from patients.

5.5. Intestinal motility and its connection with the intestinal microbiota

In the preclinical setting, it has been shown that the implementation of peristaltic modules in gut-on-a-chip models can significantly modify the outcome of epithelial differentiation, the quality of co-culture with pathogenic or symbiotic microorganisms [102], and ultimately the intestinal barrier function and immune-inflammatory response [103,104]. A prime example of this is the work by Grassart et al., who elegantly demonstrated how the invasive properties of *Shigella flexneri* are strongly influenced not only by epithelial morphology (presence of crypts) but also by physiological stimuli present in the intestine, such as peristalsis and shear stress [101]. Notably, this kind of system can be implemented with an endothelial cell layer to mimic the gastrointestinal microvascular compartment and increase physiological relevance for specific applications [105].

Since dysmotility in the gut often arises not merely from impaired muscular contraction but from dysfunction of the enteric nervous system (ENS), models that integrate neuronal components are particularly attractive for future SpA-oriented applications. A step-wise differentiation process for PSCs has been used to generate innervated intestinal organoids containing epithelium, neurons, and glial cells within a supporting mesenchyme [106–108], whereas gut-on-chip platforms can incorporate peristaltic-like mechanical deformation, flow, and—when needed—an endothelial compartment [101,102–105]. In the context of a future SpA-derived gut-on-a-chip strategy, these elements could be combined in a modular manner by coupling patient-derived intestinal epithelium to enteric neuronal/glial components and then challenging the system with defined microbial communities, candidate pathobionts, inflammatory cytokines, or metabolite perturbations.

In such a framework, the key objective would not be to assume a pre-existing neuromuscular lesion in SpA, but to test whether neuromuscular inputs materially modify epithelial and host–microbiota phenotypes relevant to the disease. Informative readouts would include neuronal or neuroepithelial calcium imaging to quantify activation states, peristaltic strain-dependent changes in epithelial differentiation and microbial behavior, barrier function measures such as TEER or tracer flux, and epithelial inflammatory outputs. In addition, the enterochromaffin-cell/serotonin axis is especially attractive as a translational interface, because intestinal epithelial enterochromaffin cells can act as chemosensory transducers linking luminal signals to neural pathways [221]; accordingly, future integrated models could assess serotonin release, epithelial enteroendocrine states, and downstream neuronal activation together with conventional barrier and cytokine readouts. This type of module would make the neuromuscular dimension of the SpA-on-chip vision more experimentally actionable while appropriately reflecting the still limited disease-specific evidence base.

5.6. Gut mucosa immune system and immune cells priming and recirculation

PDOs are increasingly used in immunology research to study how the intestinal immune system interacts with the IECs, both in healthy and disease states. Different strategies have been proposed to implement epithelial and immune cells in the same experimental setup [120,121].

Recently, Recaldin et al. elegantly demonstrated the establishment of human intestinal immuno-organoids based on the self-organization of human epithelial organoids and autologous tissue-resident memory T cells. In their model, T cells are integrated within the epithelium, and, implementing transcriptomic studies, the authors were able to study the functional aspects of immune cells (including migration and adherence) and their interaction with epithelial cells [122]. Notably, monitoring of the fate of these resident T cells, the emergence of a subtype of immune cells (in this case intraepithelial $CD8^+$ with cytotoxic capabilities) was identified as a major determinant of intestinal inflammation, and the Rho pathway was identified as an effective therapeutic target in vitro for immuno-modulation. This study provides an important framework for BTMs in studying immune responses in various settings, potentially including the priming mechanisms of SpA-relevant Th17 cells associated with the intestinal mucosa.

Of interest, Zhang et al. were able to incorporate patient-derived epithelium, immune cells (including antigen-presenting cells and $CD4^+$ naive circulating T cells), and oxygen-sensitive commensal bacterium *Faecalibacterium* in a long-term co-culture system [123]. This type of setup allowed the authors to appreciate the contribution of each individual cross-talking element within the system on the inflammatory response measured in terms of cytokine secretion and transcriptional changes in the intestinal epithelium.

The implementation of sophisticated microfluidic systems has been successfully used to allow the continuous recirculation of T cells and other immune cells to study their dynamics of penetration and interaction with tumor tissues [124,125]. This concept is of particular interest because it could potentially be successfully applied in the gut-joint model to study the recirculation and homing of immune cells between tissues where the immune cells are usually primed such as intestinal mucosa or other epithelial interfaces of interest, such as the epidermis in PsA - and peripheral tissues targeted by inflammation, including the synovium, tendons, and entheses, and the ocular vascular compartment.

Finally, while less relevant for the purpose of the study of intestinal mucosa, the development of immune-specialized BTMs for translational purposes, including modeling of the adaptive immune system and lymphoid organs biology, is under development [225–227].

6. Conclusions and future perspectives

The conceptualization of the gut-joint axis, emphasizing the role of the intestinal mucosa as a potentially central site that may contribute to or trigger the pathogenesis of SpA, represents a major breakthrough in rheumatology research with a potentially high impact in the clinical and therapeutic fields. Embracing this axis requires acknowledging the profound multifactorial pathogenesis of the disease. As recently conceptualized, accurately deciphering SpA necessitates integrating its distinct inflammatory, structural, and bioenvironmental components — most notably, the complex involvement of the intestinal interface [18]. It is precisely this multidimensional biology that traditional, reductionist preclinical models fail to adequately capture. Consequently, the overarching rationale for transitioning to advanced, human-based BTMs lies in their unique, scalable capacity to simultaneously incorporate and cross-examine these diverse pathogenic elements within a highly controlled, patient-specific platform. Organoids, OoCs, and hybrid co-culture systems integrating human-derived cells are rapidly evolving preclinical models for translational science and have reached a sufficient degree of maturity for use in several pipelines. Implementation of these models in immuno-rheumatology studies and specifically in the SpA

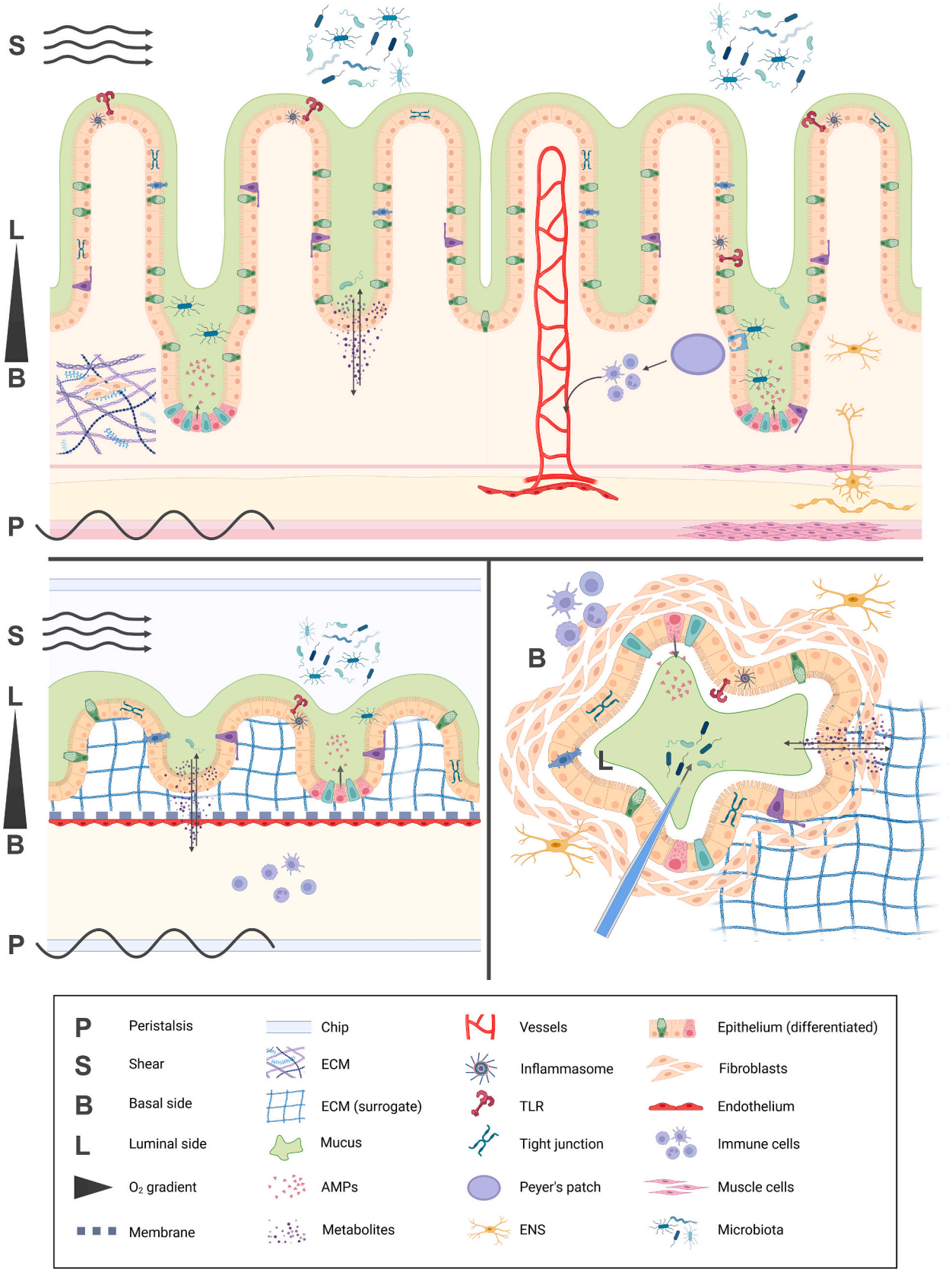

Fig. 3. Key anatomo-functional elements of gut mucosa involved in SpA pathogenesis to model through Bioengineered Tissue Models. The main types of BTMs, such as intestinal organoids (lower right panel) and gut-on-a-chip systems (lower left panel), are capable of recapitulating the intestinal mucosa of patients with Spondyloarthritis (upper panel). ECM Extracellular matrix; AMP Anti-microbial peptide; TLR Toll-like receptor; ENS Enteric nervous system. Created with BioRender.com

Table 2
Comparative overview of the main BTMs applicable to intestinal modeling in SpA.

BTM class	Main strengths	Main limitations	Typical informative readouts	Highest-value applications in SpA
3D adult stem cell-derived intestinal organoids/patient-derived organoids	Preserve donor genetic and epigenetic context; support multilineage epithelial differentiation; expandable and biobankable; amenable to perturbation studies and gene editing	Enclosed lumen limits direct apical access; lack native immune, vascular, neural, and flow-related inputs unless further engineered; donor- and batch-dependent variability requires standardization	Cell-type composition, bulk or single-cell transcriptomics, secretome, antimicrobial peptide production, innate epithelial signaling, drug response	Epithelial-intrinsic abnormalities; patient-specific barrier programs; Paneth/goblet/tuft/enteroendocrine biology; genotype–phenotype studies; biomarker discovery
Organoid-derived 2D monolayers	Direct apical-basolateral access; compatible with TEER and permeability assays; easier microbial or immune challenge; suitable for microscopy and medium-throughput screening	Reduced 3D architecture and stromal context; limited mechanical cues; shorter-term modeling of complex tissue organization	TEER, FITC-dextran or tracer permeability, junctional protein localization, directional cytokine release, transport assays	Tight-junction dysfunction; polarized host–microbe interactions; rapid screening of barrier-restoring interventions; mechanistic studies of epithelial polarity and transport
Gut-on-a-chip systems	Reproduce flow, shear stress, cyclic strain, compartmentalization, and controlled oxygenation; allow endothelial integration; enable prolonged co-culture with anaerobes and dynamic sampling	Higher cost and technical complexity; lower throughput; greater need for engineering expertise; platform-to-platform standardization remains incomplete	Real-time barrier monitoring, oxygen gradients, cytokine fluxes, live imaging, microbial viability/composition, metabolomics, multi-omics	Dysbiosis modeling; mucosa-associated microbiota; mucus dynamics; epithelial–vascular interface; anaerobe-dependent host–microbe studies; dynamic pharmacology
Hybrid co-culture systems (static or microfluidic; epithelium + immune cells and/or microbiota)	Modular and reductionist; flexible experimental design; facilitates causal dissection of epithelial–immune–microbial crosstalk	Often simplified architecture; may rely on immortalized cell lines; limited duration and physiological complexity compared with chips	Cytokine secretion, immune-cell activation, bacterial adherence/invasion, mucus thickness, epithelial cell death, targeted pathway perturbation	Candidate pathobiont testing; immune priming studies; mechanistic validation of specific pathways before deployment in more complex BTMs
Advanced integrative platforms (immuno-organoids, innervated organoids, multi-organ-on-a-chip)	Incorporate tissue-resident immunity, ENS, or inter-organ communication; closest approximation to the gut-joint axis concept	Highest complexity; limited standardization; lower scalability and accessibility	Immune-cell localization and phenotype, neuronal activity/motility-related outputs, cross-tissue mediator exchange, integrated multi-omics	Th17/Tissue resident immune cells-associated priming; motility-linked dysbiosis; future gut-joint/gut-skin-joint modeling; ex vivo precision medicine workflows

Note: Representative intestinal BTMs discussed in this review span complementary levels of complexity, including 3D intestinal organoids / PDOs [77,81–89], organoid-derived 2D monolayer or Transwell-type barrier systems [84–88], static or semi-dynamic hybrid co-culture models integrating epithelial, microbial, and/or immune components [78,122], microfluidic gut-on-a-chip platforms for host–microbiota and barrier studies [101,195,203,204,79,91,105], mucus-specialized chip systems [92–94], and advanced integrative platforms incorporating immune or neuro-enteric elements, such as immune-competent gut microphysiological systems [123,214] and ENS-containing intestinal tissues/organoids [106–108]. Unless explicitly labeled as SpA-derived, the platform examples and model selections in this table should be interpreted as fit-for-purpose frameworks for SpA-oriented studies rather than as already validated SpA-specific models.

field is still limited, and specific models are lacking. Consequently, much of the current rationale for applying BTMs to SpA should be viewed as a structured translational extrapolation from IBD, infection biology, cancer, and broader mucosal immunology, rather than as a field already supported by extensive disease-specific validation. Here, we showed that these models are promising in modeling the intestinal mucosa in SpA, underlining how relevant setups have been exploited for each key physioanatomical and functional aspect [Fig. 3].

As part of a comprehensive modeling of the anatomical and functional network of the intestinal mucosa in SpA, different types of BTMs should ideally be integrated depending on the specific research question. As a general framework, organoids could be used primarily as a source of patient-derived cellular material, retaining genetic and epigenetic information while enabling expansion, biobanking, and epithelial-focused modeling; OoCs could serve as advanced systems for reproducing physiologically relevant environmental cues and supporting complex co-culture with intestinal microbiota and immune cells; and targeted static or microfluidic co-culture systems could provide complementary reductionist settings for mechanistic dissection [Tables 2 and 3].

While BTMs now exhibit remarkable fidelity in replicating key aspects of mucosal physiology, they still face challenges that provide important avenues for refinement. Beyond biological complexity, practical aspects such as cost, scalability, availability, and standardization also shape the current landscape of BTMs technologies. Generating and maintaining some of these systems often requires specialized infrastructure, expensive reagents, and technical expertise, which can limit widespread accessibility and curb their adoption outside specialized centers. Variability in culture conditions and biomaterials can affect reproducibility, underscoring the need for harmonized protocols and shared reference standards. Moreover, the push for industrialization and broader market penetration is in early stages, with regulatory policies and commercial leveraging still evolving [229].

Encouragingly, many of these challenges are now being addressed. Guidelines for laboratory procedures, biobanking workflows, and quality control are under development (e.g., via ISO/TC 276), with institutional roadmaps addressing device materials, interfaces, flow conditions, terminology, and performance benchmarks crucial for interoperability and regulatory acceptance [230,231]. Standalone modular chip platforms that are more cost-effective and user-friendly are now available from several suppliers [232]. Biobanking initiatives and automation tools are enhancing throughput and cross-institutional sharing of organoids [233], including pioneering work embedding patient-centered decentralized biobanking, which could significantly democratize participation and equity in BTMs research is ongoing as well [234]. Thus, while economic and logistical barriers remain, the innovation trajectory strongly suggests that BTMs are transitioning from niche, resource-intensive tools toward standardized and widely deployable platforms for translational research.

Besides the specific focus of this review, there are some additional elements worth mentioning in support of the prompt use of BTMs and their future development in the SpA field.

As anticipated, genetics is central in determining the onset and expression of SpA. HLA-B27 is the major determinant and is supposed to have a direct pathogenic role through misfolding or immunologic mechanisms. Other genetic candidates, such as single-nucleotide polymorphisms in *ERAP1*, could also be important [235]. The most evident advantage of using these ex vivo models is the possibility of retaining the

Table 3
Harmonized roadmap for selecting and validating BTMs for intestinal modeling in SpA.

SpA-relevant biological question	Best-fit model (and main references)	Recommended inputs	Core readouts	Validation against human specimens	Provisional decision rule for escalation / drug triage
Does the overall patient-derived microbiota drive epithelial dysfunction or inflammatory activation?	Anaerobic gut-on-a-chip with PDO-derived epithelium; optional endothelial compartment [195,204,105]	Patient ileal/colonic PDOs; paired fecal and, where feasible, mucosa-associated microbiota from SpA and matched controls; optional autologous myeloid cells	Oxygen-gradient verification, microbial viability/composition, TEER, FITC-dextran flux, epithelial cytokines, bulk/scRNA-seq	Concordance with stool/biopsy microbiome profiles, biopsy histology, fecal calprotectin, LBP/iFABP, mucosal inflammatory signatures	Escalate or prioritize interventions only if barrier/inflammatory phenotypes are reproducible across independent donors and rescued by the candidate intervention without collapse of microbial viability
Is a specific strain or defined bacterial consortium causally pathogenic?	Reductionist co-culture [78] or gut-on-a-chip for oxygen-sensitive organisms [101,203,204]	PDO-derived monolayers or chip epithelium; single strain or defined consortium selected from SpA datasets/culturomics; optional innate immune cells	Adherence/invasion, epithelial cell extrusion, inflammasome/TLR activation, cytokines, metabolite production	Presence/abundance of the same strain(s) in patient stool/biopsy datasets; biopsy evidence of bacterial encroachment or inflammation	Advance only if the phenotype is reproducible, directionally consistent with patient data, and attenuated by bacterial inactivation, depletion, or pathway blockade
Is there an epithelial-intrinsic barrier defect in SpA?	Organoid-derived 2D monolayers / Transwells [84–88]; chip if dynamic flow is needed [79,91]	SpA and control PDOs, with or without TNF-α / IL-17 A / IFN-γ challenge; microbial supernatants or metabolites as secondary perturbations	TEER, tracer flux, ZO-1/OCLN/CLDN localization, polarity markers, apical/basolateral cytokine release	Biopsy TJ protein expression/localization; permeability-associated blood markers; endoscopic/histologic inflammation	Prioritize compounds only when both functional and structural barrier rescue are observed in donor-level replication, ideally with an orthogonal second assay
Are mucus barrier defects biologically relevant in SpA?	Mucus-competent gut-on-a-chip [92–94] or goblet-supporting organoid/co-culture system [78,89]	PDO-derived epithelium enriched for goblet differentiation; patient microbiota or defined strains; optional inflammatory cytokine challenge	Mucus thickness, penetrability, bilayer organization, goblet-cell differentiation, bacterial localization relative to epithelium	Mucin/goblet-cell histology from biopsies; microbial encroachment patterns; inflammatory phenotype of matched tissue	Escalate if the platform detects reproducible mucus defects that can be restored without worsening epithelial inflammation or viability
Do SCFAs or tryptophan/indole metabolites restore homeostasis?	Monolayers for barrier assays [84,88]; PDOs for lineage/cell-state analysis [218,219,64]; gut-on-a-chip for dynamic host–microbe metabolism [62,63]	Defined SCFAs or indoles; metabolite-conditioned microbial supernatants; SpA and control PDOs; inflammatory challenge where relevant	TEER, tracer flux, cytokines, scRNA-seq cell states, mitochondrial readouts in LGR5-rich compartments, Paneth-cell AMP programs	Stool/serum metabolomics; biopsy expression of AhR/IL-10R1, AMP, and inflammatory signatures	Advance candidate metabolites or microbiota-modifying drugs only if protection is dose-responsive, donor-reproducible, and reflected in both barrier and molecular endpoints
How are Paneth-cell or epithelial antimicrobial-defense programs altered?	Differentiated small-intestinal PDOs / enteroids [77,81–83]	SpA and control small-intestinal PDOs; cytokine or microbial challenge; optional IL-22 or metabolite perturbation	DEFA5, LYZ, REG-family AMP programs, Paneth-cell abundance/function, epithelial stress signatures	Biopsy AMP expression and crypt histology; associations with mucosal inflammation and microbiota composition	Prioritize interventions that restore antimicrobial-defense programs without inducing nonspecific epithelial stress or loss of stem-cell fitness
How does the mucosal immune compartment contribute to pathogenic priming?	Immuno-organoids [122] or immune-competent gut-on-a-chip [123]	PDOs plus autologous tissue-resident or circulating immune subsets (e.g., CD4+ T cells, APCs, Tissue resident immune cells), with or without defined bacteria	Immune-cell migration/adherence, cytokines, epithelial–immune crosstalk, scRNA-seq / spatial phenotyping	Biopsy immune infiltrates, mucosal cytokine profiles, paired blood immunophenotyping	Advance immunomodulatory candidates only if they suppress pathogenic epithelial–immune programs while preserving basic tissue viability and barrier function
Does the gut-vascular interface contribute to systemic inflammatory propagation?	Vascularized gut-on-a-chip or advanced multi-compartment chip [105,107,228]	PDO-derived epithelium plus endothelial layer; microbial products or bacteria; optional immune-cell flow-through	Endothelial permeability, PV1/JAM-A/VE-cadherin-related readouts, translocation, immune-cell adhesion/migration	Biopsy vascular-barrier markers; circulating LPS/LBP, sCD14, iFABP; systemic inflammatory markers	Escalate to more complex platforms only when epithelial findings suggest a dissemination mechanism that cannot be resolved in epithelial-only systems
Do motility and ENS-related cues reshape host–microbe interactions?	Peristaltic gut-on-a-chip [101,102–105] or ENS-containing organoids [106–108]	Mechanical strain and flow, with or without PSC-derived ENS components; defined microbiota or metabolites	Calcium imaging, serotonin/EC-cell coupling, TEER, tracer flux, epithelial cytokines, microbial spatial behavior, differentiation state under peristaltic strain	Where available, biopsy ENS markers and clinical dysmotility correlates; indirect association with microbiota signatures	Use as a second-line mechanistic platform when the hypothesis specifically depends on physical or neuro-epithelial regulation rather than routine first-pass screening

Note: Across all rows, minimum harmonization elements should include anatomical sampling metadata, predefined organoid passage windows, matrix/media lot tracking, lineage and polarity QC, oxygen verification when anaerobes are used, donor-level biological replication, and prespecified alignment of ex vivo endpoints with human reference biomarkers. Unless explicitly labeled as SpA-derived, the platform examples and model selections in this table should be interpreted as fit-for-purpose frameworks for SpA-oriented studies rather than as already validated SpA-specific models.

disease- and patient-specific genetic background. Furthermore, BTMs provide a powerful backbone for the use of genetic manipulation tools, including the CRISPR-Cas system, transposon mutagenesis, and siRNAs that could be used to research the effect of known genetic variants identified by Genome-wide association studies and determine valuable disease targets [174]. Epigenetic factors are also increasingly recognized as determinants in the disease onset and expression and can be assessed in BTMs models through specific assays [236,237].

Importantly, given its multifaceted nature, SpA should be considered a systemic disease. Optimal human preclinical modeling of the disease thus requires creating and functionally coupling valid models for each of the systems and tissues primarily involved in the immuno-inflammatory process. This is an attainable goal due to the current technological evolution of BTMs [228], allowing unparalleled flexibility and control

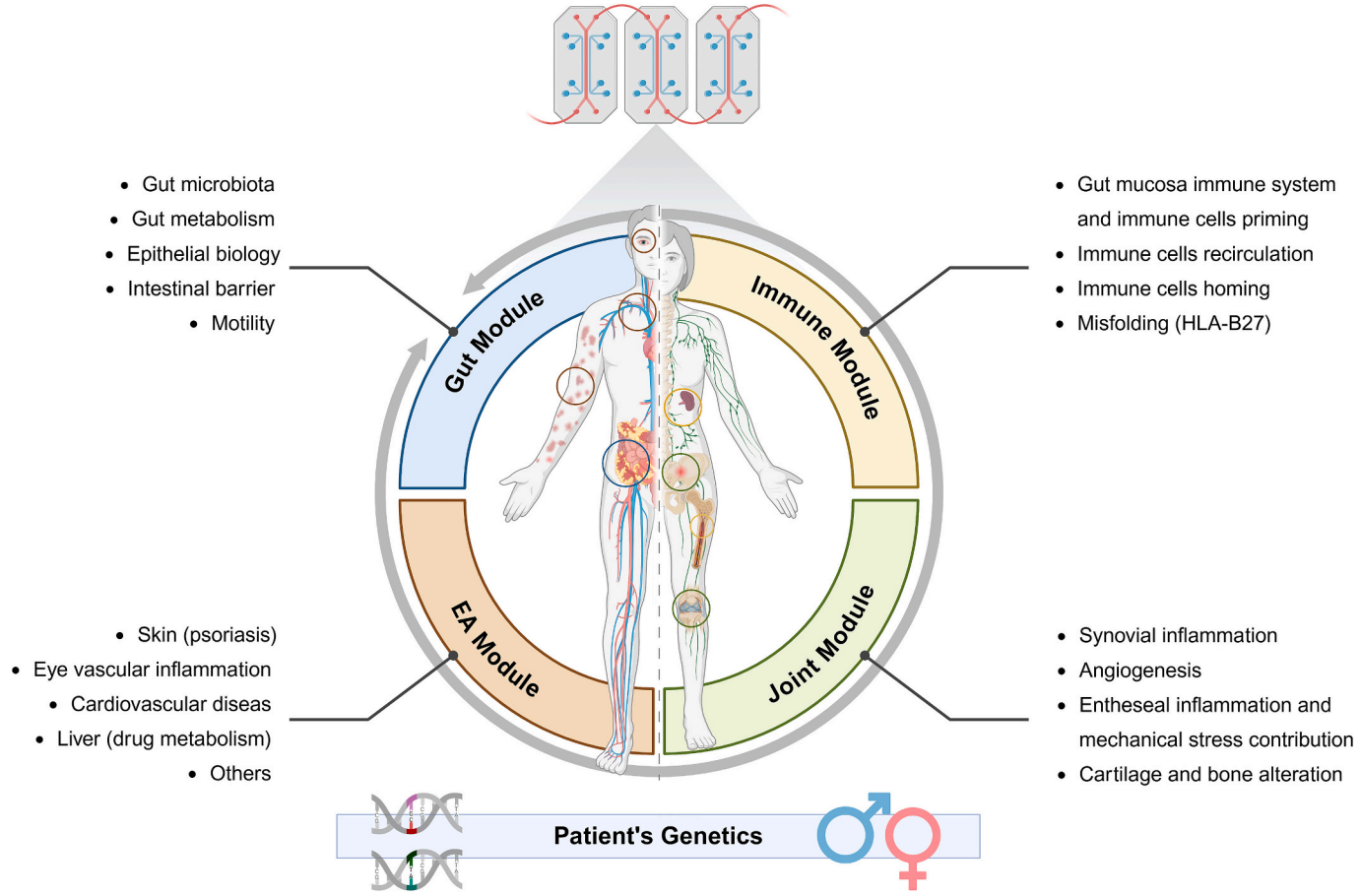

Fig. 4. Key aspects to recapitulate for a comprehensive Spondyloarthritis preclinical model (SpA-on-a-chip) based on BTMs. A future SpA preclinical platform based on the gut-joint pathophysiological model should integrate several relevant intercommunicating tissue modules. EA Extra-articular. Created with BioRender.com

over traditional models. Therefore, future development in this field should aim at developing a comprehensive "SpA-on-a-chip" platform based on the pivotal premise of the gut-joint axis. Consequently, the possibility of having a faithful in vitro "replica" of the patient available to test an array of molecules with potential therapeutic effects represents an appealing, albeit currently theoretical, iteration of the concept of personalized therapy [238] [Fig. 4].

While this review focuses specifically on the intestinal mucosa, a detailed examination of BTMs for the downstream musculoskeletal targets of SpA — such as the synovium, cartilage, and particularly the enthesis — falls outside its current scope. However, realizing a functionally relevant "SpA-on-a-chip" will necessitate a carefully staged, modular approach. The first stage requires the independent development and robust validation of the individual tissue modules (e.g., the gut barrier and the entheseal interface). The second stage will involve the microfluidic coupling of these platforms into a multi-organ system. This multi-organ integration is critical, as it will finally allow researchers to directly track the dynamic, systemic crosstalk hypothesized in the gut-joint axis, enabling the observation of how gut-primed immune cells, microbial metabolites, or pro-inflammatory cytokines migrate through a simulated vascular network to trigger inflammatory or osteoproliferative responses in the joint module.

Finally, it must be emphasized that failure in the shift from preclinical to clinical phase, with the dropout of numerous molecules, represents a major challenge in drug development. The immunorheumatology field makes no exception, while heterogeneity of clinical phenotype of individual systemic diseases is an additional obstacle and may result in failure of late-stage trials [239]. In the specific context of SpA, the use of BTMs for patient stratification and ex vivo prediction of therapeutic responses remains largely speculative. Before these bioengineered platforms can be reliably translated to inform clinical decision-making or de-risk late-stage trials, they face significant challenges. They necessitate rigorous, large-scale validation using SpA-specific experimental settings to definitively prove that in vitro responses accurately mirror in vivo clinical outcomes for individual patients. The ultimate goal of preclinical development remains to provide therapeutic approaches transferable to clinical practice. In this regard, BTMs offer tangible opportunities to enhance disease modeling and drug discovery, and ultimately increase the chance to offer patients individualized and effective medical care.

7. Search strategy and selection criteria

To provide a comprehensive overview of the current landscape, we conducted a literature search using PubMed/MEDLINE, Embase, and Scopus databases for articles published up to August 2025. The search strategy employed combinations of keywords and their variations (including MeSH terms where applicable) to capture broad concepts. Key terms included:

- Disease: "Spondyloarthritis" (OR "Ankylosing Spondylitis", "Psoriatic Arthritis", "Spondyloarthropathy", "Reactive Arthritis", "Enteropathic Arthritis");
- Physiopathology: "Gut-joint axis", "Intestinal Mucosa" (OR "Gut barrier", "Intestinal epithelium", "Leaky gut"), "Microbiota" (OR "Microbiome", "Dysbiosis");
- Models: Organoids" (OR "Enteroids", "Colonoids"), "Organs-on-a-chip", "Gut-on-chip", "Microphysiological systems").

We utilized truncation to include all grammatical variations. Given the review's focus on translational validity, we applied a strict preference for human-derived data and in vitro models utilizing human cells. Animal studies were referenced mainly to provide historical context or to illustrate specific limitations in replicating human mucosal physiology. We prioritized English-language articles published within the last 15 years to ensure coverage of recent technological breakthroughs, while manually screening reference lists of eligible articles to identify seminal works outside this window.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data are available in the published articles included in this review.

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