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Epithelial and microbial determinants of colonic drug distribution

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ABSTRACT

A dynamic epithelium and a rich microbiota, separated by multi-layered mucus, make up the complex colonic cellular environment. Both cellular systems are characterized by high inter- and intraindividual differences, but their impact on drug distribution and efficacy remains incompletely understood. This research gap is pressing, as, *e.g.*, inflammatory disorders of the colon are on the rise globally. In an effort to help close this gap, we provide considerations on determining colonic epithelial and microbial cellular parameters, and their impact on drug bioavailability. First, we cover the major cell types found *in vivo* within the epithelium and microbiota, and discuss how they can be modeled *in vitro*. We then draw attention to their structural similarities and differences with regard to determinants of drug distribution. Once a drug is solubilized in the luminal fluids, there are two main classes of such determinants: 1) binding processes, and 2) transporters and drug-metabolizing enzymes. Binding lowers the unbound intracellular fraction ($f_{u,cell}$), which will, in turn, limit the amount of drug available for transport to desired sites. Transporters and drug metabolizing enzymes are ADME proteins impacting intracellular accumulation (Kp). Across cell types, we point out which processes are likely particularly impactful. Together, $f_{u,cell}$ and Kp can be used to describe intracellular bioavailability (F_{ic}), which is a measure of local drug distribution, with consequences for efficacy. Determining these cellular parameters will be beneficial in understanding colonic drug distribution and will advance the field of drug delivery.

1. Drug delivery to (part of) a supraorganism

The human body is a supraorganism, containing an estimated 1.3-fold as many microbial cells as human cells (Sender et al., 2016). Though microbial exposure is highest at epithelia, microbes have even been detected in the blood of healthy individuals (Velmurugan et al., 2020). This suggests that drugs circulating anywhere in the body are continually exposed to both cells within human organs and a collective microbial "organ", with implications for drug delivery as a whole. Considering microbial impacts is, however, most relevant where local organ and drug exposure to the microbiota are highest, namely in the colon (Sender et al., 2016). Information on how drugs distribute in the heterogeneous colonic cellular environment is limited. In this review, we identify major research gaps regarding colonic cellular parameters that describe drug distribution and efficacy, such as intracellular bioavailability (F_{ic}). Filling in these gaps will notably contribute to the growing need for efficacious pharmacotherapy of inflammatory and other colonic disorders.

2. Major colonic cell types

We first present the major colonic cell types in both the epithelium and microbiota. We then discuss how they can be modeled *in vitro* to determine cellular parameters.

2.1. Epithelium: Colonocytes and goblet cells

2.1.1. Colonic epithelium

The mucosal epithelium is an undulating monolayer at the luminal surface of the colonic tube (Fig. 1). Tight junctions seal this physical barrier against the lumen (Artursson et al., 2001). Both water and nutrient absorption, and the production of mucus are key tasks that the epithelium fulfills (Dutton et al., 2019). It derives from crypt-base columnar cells. These stem cells give rise to absorptive and secretory cell types, with various cell types and transition states existing along the crypt-surface axis. Less abundant secretory cell types are deep secretory cells, enteroendocrine cells, and tuft cells (Fig. 1). They secrete, *e.g.*, antimicrobial substances, hormones, and interleukins, respectively,

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which are important in regulating gut homeostasis and immune function. Two cell types, namely absorptive colonocytes and mucin-secreting goblet cells (Fig. 2) (Beumer and Clevers, 2021), however, make up most of the surface. Together, they account for ~93% of the monolayer. Averaging about 20%, goblet cell abundance increases from the proximal to the distal colon for a longitudinal increase in mucus production (Dutton et al., 2019). Multi-layered colonic mucus is a dynamic and heterogenous continuum. Though the transition between layers is fluid and thicknesses vary (Nyström et al., 2021), two main layers can still be discriminated (Fig. 1). The inner mucus is intimately linked to the epithelium, as it remains tethered via transmembrane mucins in the glycocalyx (Johansson and Hansson, 2016). Conversely, the outer mucus is non-adherent and subject to substantial remodeling by colonizing microbes (Li et al., 2015). Outer mucus can therefore be considered as a partially "microbial" layer despite its epithelial origin.

2.1.2. Colonic epithelial in vitro models

The short epithelial cell lifespan, and hence high turnover, has historically made the intestinal epithelium difficult to accurately model *in vitro*. Tumor-derived cell lines such as Caco-2 form tight monolayers and address the longevity issues. These cell lines have been used in various cultures and co-cultures to model not only permeability, but also immunological aspects of the colonic environment (Kleiveland, 2015). However, they do not capture different colonic cell types, and have poorly representative proteomes (Ölander et al., 2016). Hence, for the determination of physiologically relevant cellular parameters, more accurate *in vitro* models are needed. In this regard, colonoids grown from adult colonic stem cells show great promise. Recently introduced as a more *in vivo*-like alternative, this technology allows epithelial cell layers to be maintained in long-term 3D or 2D culture without the need for using tumor-derived cells with compromised genome integrity (Pleguezuelos-Manzano et al., 2020). Colonoids may even be grown in co-cultures with gut microbes (Sasaki et al., 2020). However,

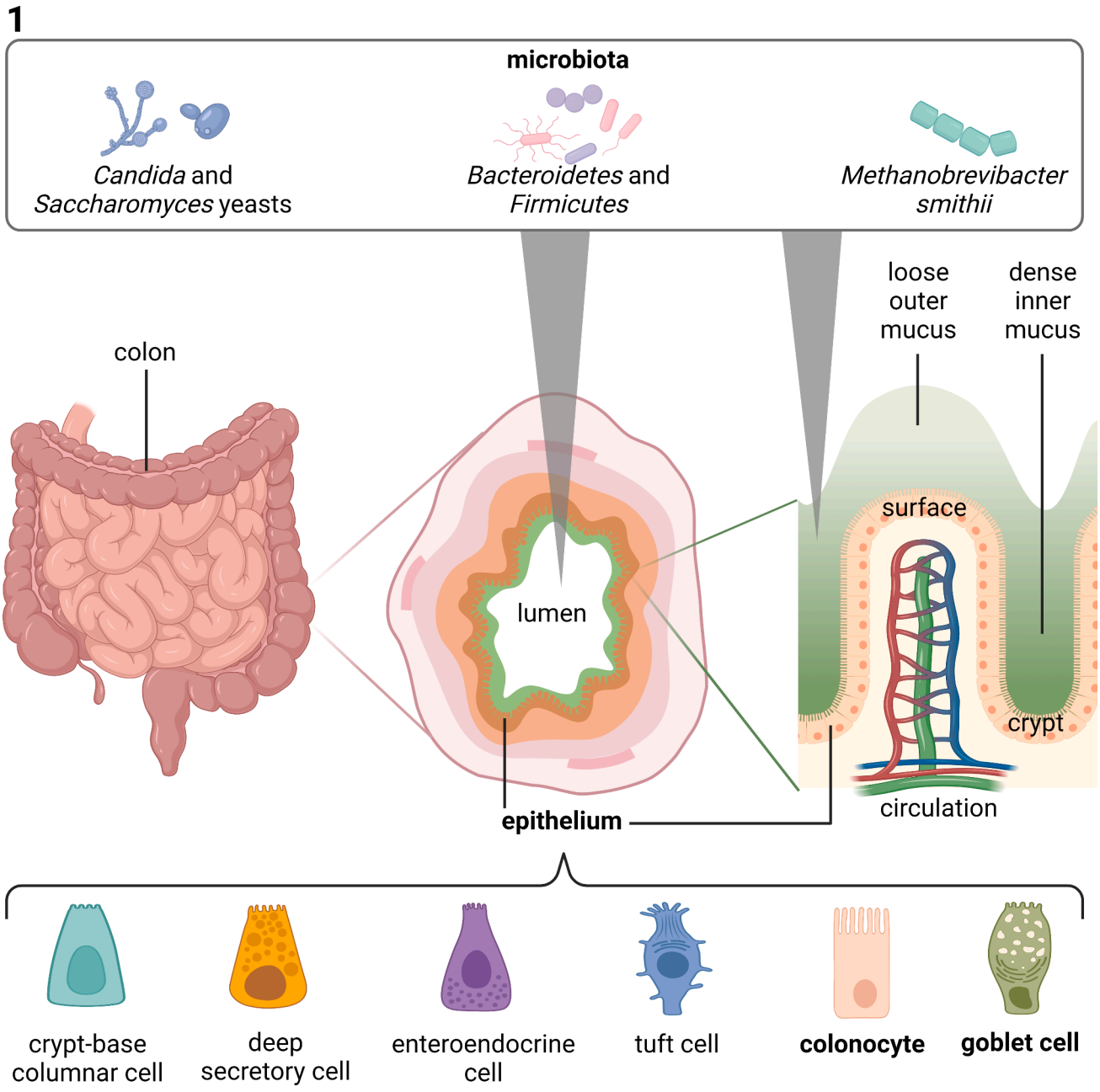

Fig. 1. Overview of colonic anatomy and the major epithelial and microbial cell types.

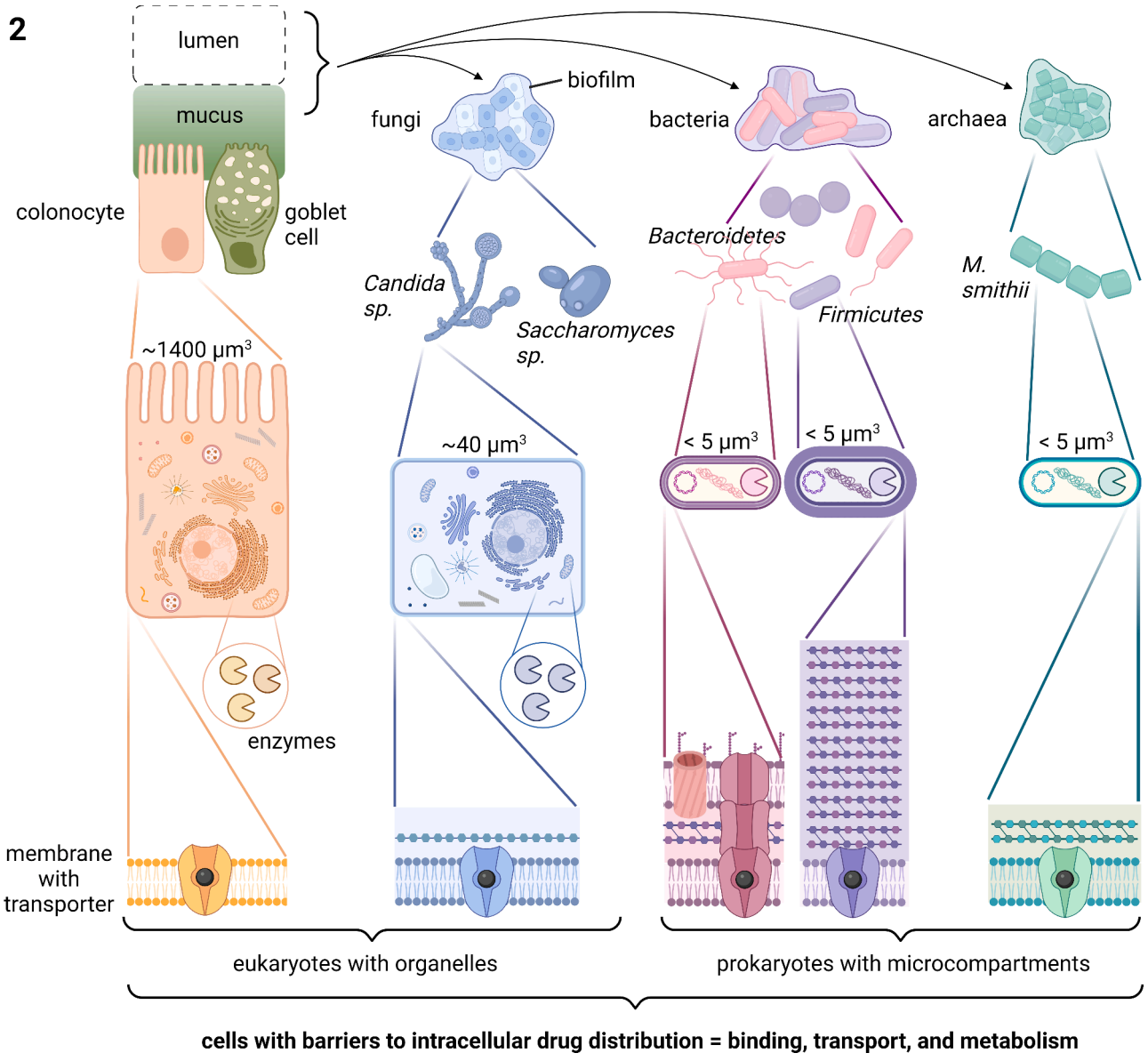

Fig. 2. Localization and structural features of cell types in the colon. Approximate cellular volumes were estimated from (Jorgensen et al., 2002; Levin and Angert, 2015; Miquel et al., 2013; Samuel et al., 2007; Wiśniewski et al., 2015).

reproducibly differentiating stem cells into defined proportions of colonocytes and goblet cells remains a challenge. Likewise, colonoid proteomes and how they are impacted by culture conditions are poorly characterized to date. Further work in these areas will benefit the validity assessment of cellular parameters determined in colonoids.

2.2. Microbiota: Mostly bacteria

2.2.1. Colonic microbiota

The outer mucus and lumen are inhabited by trillions of microbes (Fig. 1) (Li et al., 2015; Sender et al., 2016). Microbial cell types include both eukaryotes (fungi) and prokaryotes (bacteria and archaea) (Hoffmann et al., 2013; Rinninella et al., 2019). Whether containing one or many species, microbes preferentially form metabolically synchronized and interconnected composites, including biofilms and planktonic luminal communities (Fig. 2) (Motta et al., 2021). As a simplified model of this intricate ecosystem, we highlight key gut microbial types based on relative abundance. The most abundant fungi in the human gut are facultative anaerobic *Candida* and *Saccharomyces* yeasts (Hoffmann et al., 2013). Growing in hyphae or buds, they typically account for less than 1% of the gut microbiome (Arumugam et al., 2011). Bacteria are the most abundant component of the microbiome, represented by many phyla. Gram-negative *Bacteroidetes* and Gram-positive *Firmicutes* make up around 90% of the gut microbiome (Rinninella et al., 2019); they, *e.g.*, supply the epithelium with short-chain fatty acids (SCFAs) as nutrients, synthesize key vitamins, and provide colonization resistance against pathogenic microbes (Motta et al., 2021). The archaeon *Methanobrevibacter smithii* represents up to 10% of the gut microbiota (Miller and Wolin, 1986). It produces methane, which impedes the distribution of water-soluble drugs.

2.2.2. Colonic microbiota ex vivo models

In vitro microbiota models should ideally recapitulate as many aspects of the native colonic microbial environment as possible to encourage native-like growth (Stewart, 2012). Maintaining anaerobic conditions to mimic the gut is essential, whether simplified models such as individual strains or complex fecal slurries are used (*e.g.*, (Klünemann et al., 2021; O'Donnell et al., 2016)). Isolation and culturing of

individual strains facilitates study of microbial processes, but also neglects network interactions among species. Such interactions constitute a vital aspect of microbial habitats, and some microbial strains are unculturable unless co-cultured with other species (Stewart, 2012). Fecal slurries, for which standardized approaches have been developed (O'Donnell et al., 2016), address this issue by sampling from the whole fecal microbiota. Still, even in this more complex model, key aspects of the native gut environment such as fluctuating water content, nutrient gradients, epithelial secretions, local microniches, and an intact 3D biogeography are missing. Because stool is sampled from the end of these gradients, *i.e.* from the rectum, fecal slurries also do not fully represent proximal colonic microbiota. While several other gut microbial sampling methods are available, they entail their respective advantages and disadvantages (Tang et al., 2020). However, one disadvantage current standard sampling methods have in common is that they at least temporarily breach the gut microbes' anaerobic environment. Use of adapted colonoscopic sampling protocols may solve this issue. Recently, luminal contents have been colonoscopically sampled without substantially perturbing the colon segment in question (Lemmens et al., 2021a). Such an approach could likely permit future microbial sampling from a range of colonic sites, while maintaining anaerobic conditions and allowing mucus access. Despite current sampling limitations, strides have recently been made in standardizing the *in vitro* assessment of clinically relevant bacterial drug degradation using simulated colonic bacteria (Vertzoni et al., 2018). If this and other *in vitro* models prove similarly useful in modeling drug distribution to and within the microbiota, the question of the overall representativeness of current models may be resolved.

3. Colonic determinants of cellular drug distribution

Most drug targets are proteins, located intracellularly. To successfully reach and bind an intracellular target in the colonic epithelium or deeper tissues, a drug must evade cellular barriers to drug permeation – not only within the epithelium, but also in the microbiota. Uptake transporters are positive effectors impacting permeability. We summarize both barriers and positive effectors as determinants of cellular drug distribution. These determinants can be classified into binding processes directly impacting intracellular unbound concentrations ($f_{u,cell}$), and drug-transporting proteins and drug-metabolizing enzymes (DMEs), which primarily impact total cellular accumulation (Kp). Lipids, though also impacting Kp via passive lipoidal permeability, are covered under binding processes. Narrowing down which types of determinants are particularly impactful, both in each cell type and the colonic environment as a whole, will aid in describing drug distribution.

3.1. Binding processes impact free drug concentration

Depending on their own molecular properties, drugs may substantially bind to polysaccharides, poly-organized lipid structures, proteins, and/or nucleic acids (Fig. 3). Binding is pH- and ionic strength-dependent. It occurs both intra- and extracellularly. In the colon, a notable extracellular source of potential binding are food residues. Food residues are, however, beyond the scope of the present review focusing on barriers created by cellular constituents. Unwanted binding, regardless of its nature, renders drugs (at least temporarily) unavailable for interacting with their target, thereby lowering the unbound intracellular fraction ($f_{u,cell}$). Transient, nonspecific binding occurs throughout cellular environments (Fig. 3). With regard to the colonic cellular environment, we focus on sinks. Sinks occur if large deposits of drug-binding substances (allowing for repeated nonspecific binding and therefore larger binding timescales) or major pH shifts are present (Fig. 3). All the following types of colonic sinks have in common that protein is a major constituent (summarized as total protein in Table 1). Here, we mainly focus on the non-protein constituents (if applicable) that distinguish each type of sink.

3.1.1. Glycan filters (sugars)

Glycans are main constituents of the epithelial glycocalyx, colonic mucus, microbial extracellular polymeric substances (EPSs), S-layers, and cell walls (Table 1). Though the sugar moieties and layer thicknesses vary between domains and species, glycan layers have in common that they act as molecular filters. In these filters, nanosized pores typically decrease in size from the surface inwards. Even the innermost pores are large enough to permit the passage of small molecule drugs (Boegh and Nielsen, 2015; de Souza Pereira and Geibel, 1999; Pasquina-Lemonche et al., 2020; Sleytr et al., 2014). Drugs can, however, become trapped by electrostatic interactions with negatively charged glycans. Hydrophilic drugs, especially with charges unevenly distributed throughout the molecule, are likely to be sequestered in glycan filters (Boegh and Nielsen, 2015).

3.1.2. Phospholipid membranes (lipids)

In human cells, lipids are a dominating cellular constituent (Table 1).

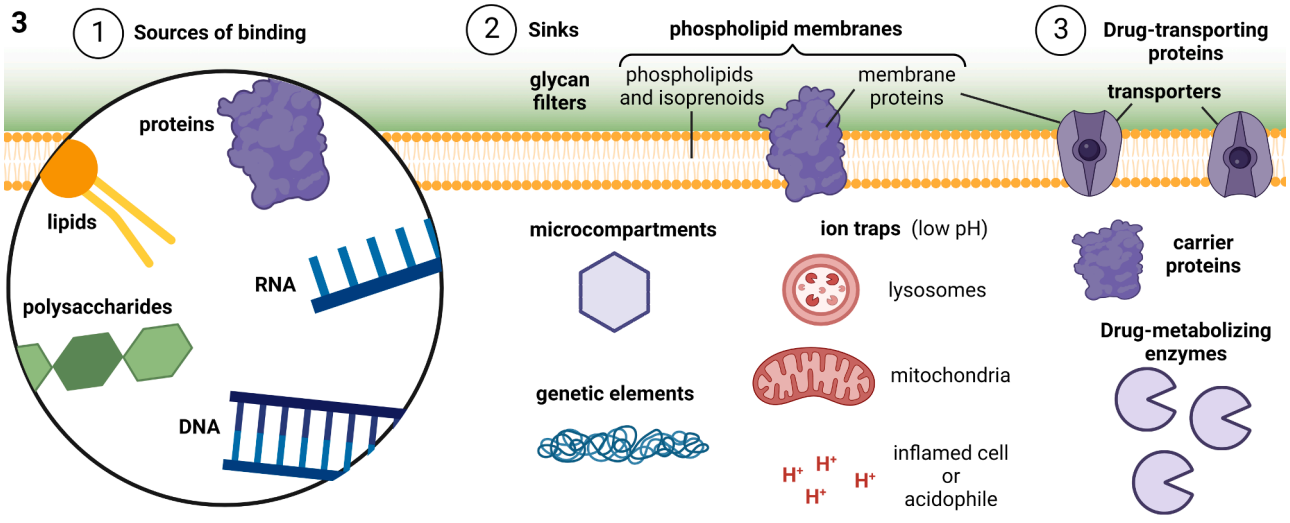

Fig. 3. Determinants of intracellular drug distribution: 1, Sources of substantial binding. 2, Sinks are comprised of one or more of these sources. 3, Drug-transporting proteins and drug-metabolizing enzymes. Colors of main constituents: lipids (yellow), proteins (purple), nucleic acids (blue), polysaccharides (green)). Ion traps shown in red.

Table 1
Overview of cellular sinks and their major constituents. Where indicated, percentages and amounts estimated per cell refer to cellular dry weight. Values are approximate. EPS: Extracellular Polymeric Substance, MP: Mannoprotein, LPS: Lipopolysaccharide, PL: Phospholipid, PC: Phosphatidylcholine, PG: Phosphatidylglycerol, PE: Phosphatidylethanolamine, C: Cholesterol, E: Ergosterol, CL: Cardiolipin, H: Hopanoids. For table references (given in parentheses), see Appendix.

Type		Epithelium		Fungi (yeasts)		Gram-negative bacteria		Gram-positive bacteria		Methanogenic gut archaea	
Nanosized glycan filters		*Mostly extracellular*	Mucins (inner mucus/ glycocalyx) (1)	*Extracellular 11–30%* (3a, b)	EPSs (2) MPs, beta-glucans, chitin	*Extracellular 6%* (3c)	EPSs (2) S-layer glycoproteins (4), LPSs, murein	*Extracellular 10–15%* (5)	EPSs (2) S-layer glycoproteins (4), lipoteichoic acid (6), murein	*Extracellular Likely similar to bacterial values*	EPSs (2) Pseudomurein (7)
PLs and isoprenoids		*13%* (3d)	PC, PE, C (8)	*4–10%* (3d)	PC, PE, E (8)	*9%* (3e)	PG, CL, PE, H (9)	*6–7%* (10)	PG, CL, PE, H (9)	*6%* (11)	Caldarchaeol, archaeol (7)
Proteins		*60%* (3d)	250 pg (3f)	*40–55%* (12)	4 pg (13)	*55–66%* (3e,14)	156 fg (3 g)	*38–71%* (14)	Likely similar values to Gram-negative	*Likely below bacterial values due to smaller average protein size* (3 h)	
Nucleic acids	**DNA**	*1%* (3d)	6.5 pg (15)	*< 1%* (3d)	0.017–0.034 pg (3i,j)	*3%* (3e)	9 fg (3 g)	*Same order of magnitude as Gram-negative* (3k)		*Same order of magnitude as Gram-negative* (16)	
	RNA	*4%* (3d)	25–30 pg (3l)	*6–12%* (3d)	0.5–1 pg (3 m)	*21%* (3e)	60 fg (3 g)	*Likely similar to Gram-negative values*		*Likely similar to bacterial values*	

Though lipophilic drugs are more likely to become sequestered, most drugs must interact with membranes to diffuse into cells. Differences in lipid composition can impact permeability and drug binding (Treyer et al., 2018). Microbial cellular lipid composition differs substantially from that of human cells (Table 1), but how this affects binding is poorly characterized. What is known is that microbes are both substantially smaller than human cells (Fig. 2) and have a lower proportion of lipids (Table 1). Overall, this suggests that microbial lipids may play a subordinate role as sinks.

3.1.3. Microbial microcompartments (protein)

Prokaryotes are generally more protein-dense than eukaryotes, unless they are exposed to substantially nutrient-poor conditions (Table 1). Their cell membranes have a greater proportion of membrane proteins than those of eukaryotes (BNIDs 106,255 and 111,959, Milo et al., 2010). Lacking organelles, they also use protein shells known as microcompartments to encapsulate metabolic processes. Examples of microcompartments are Eut and Pdu, which generate phosphorylated precursors of SCFAs from ethanolamine and 1,2-propanediol, respectively. Most presently characterized microcompartments are selectively permeable to substrates too small to be druglike. However, druglike substances can interact with the hydrophilic and lipophilic residues exposed on the large protein surface of microcompartments (Chowdhury et al., 2014). The formal proof that bacteria can function broadly as sinks (termed “bioaccumulators” by the authors) has also been linked to protein binding. Duloxetine, a serotonin–norepinephrine reuptake inhibitor, was bioaccumulated by the greatest number of species and linked to shifts in microbial metabolism and community composition (Klünemann et al., 2021). Drugs with similar propensity for microbial protein binding will also be notably distributed to colonic bacteria.

3.1.4. Intracellular genetic elements (nucleic acids)

DNA in nuclei, nucleoids, mitochondria, and plasmids can function as drug sinks, especially for drugs prone to binding polyanionic structures, or targeting nucleic acids in more selective ways (Urbinati et al., 2008). Though eukaryotes have larger quantities of DNA per cell (Table 1), it is shielded by additional membranes (Fig. 2), making it more difficult to access. Prokaryotic DNA is, conversely, quite accessible, especially as it is more frequently in an uncoiled state due to relatively shorter doubling times (BNID 103,891, Milo et al., 2010). Plasmids can introduce additional DNA into prokaryotes, which further marginally increases the total DNA content (BNID 107,527, Milo et al., 2010). The amount of RNA is also substantially higher in prokaryotes (Table 1). Notably, in the inflamed gut, substantial quantities of extracellular nucleic acids are present, stemming both from transmigrated neutrophils that die in the gut lumen (Fournier and Parkos, 2012), as well as from microbiota cells killed by the inflammatory condition. It is therefore plausible that the sink properties of nucleic acids (primarily long-lived DNA) will be most significant in the inflamed gut.

3.1.5. Ion traps (pH)

Compartments with a relatively lower pH can trap drugs via protonation, which makes them less permeable to membranes. This applies not only to acidic organelles such as lysosomes and mitochondria within colonocytes, but also to entire cells and tissues (such as in inflammatory bowel disease (Nugent et al., 2001)). Particularly, inflamed epithelial cells and acidophilic bacteria, such as those that produce SCFAs (Chowdhury et al., 2014) have a relatively lower pH, making them candidates to act as ion trap sinks in the gut.

3.1.6. Impact of sinks

The unbound fraction of a drug in a cell-representative matrix ($f_{u,matrix}$) is defined as the ratio of the drug concentration in buffer to that in matrix. It can be scaled to $f_{u,cell}$ using a dilution factor (D) (Mateus et al., 2013). To date, determinations of $f_{u,cell}$ have focused mainly on lipid binding in mammalian cells, with membrane surrogates and cell

homogenates used as matrices (Mateus et al., 2017; Nichols et al., 2022; Treyer et al., 2018). How applicable are these two methodologies to colonic cell types? Though (Nichols et al., 2022) suggests that some drugs can interact with their target even *within* membranes, mammalian $f_{u,cell}$ is typically dominated by membrane partitioning (Mateus et al., 2017). For the purpose of determining F_{ic}, lipid- or otherwise sink-bound drugs can be assumed to be unavailable for target interaction, as postulated by the free drug theory (Summerfield et al., 2022). Using defined amounts of concentrated lipids as a membrane surrogate is meaningful in cells dominated by lipids. While this, broadly speaking, is true for epithelial cells, other types of sinks tend to dominate in microbes (Table 1). The homogenate approach, essentially meaning pooling all of the sinks in a cell, seems more suitable here. However, the lipid approach cannot, and the homogenate approach likely will not, capture, *e.g.*, the mucus layer(s), potential sinks relevant for all colonic cells. Most *in vitro* systems lack fully developed mucus, as this is difficult to recapitulate, and as mucus may often (at least partially) wash away during sample preparation. Use of mucus (or mucus surrogates) as an additional matrix as described in (Witten et al., 2019) could solve this issue.

3.2. Drug-transporting proteins and drug-metabolizing enzymes impact accumulation

Transport processes (active, via solute carriers, or via carrier proteins) and metabolic modifications (via DMEs) impact the cellular accumulation of drugs (Kp). In brief, drug uptake increases Kp, whereas efflux and metabolism decrease Kp (Mateus et al., 2017; Wegler et al., 2021). To date, these processes have not been well characterized in colonic cells, partly due to a lack of representative *in vitro* cell models. We review the limited work available thus far and provide a perspective on colonic Kp value assessment.

3.2.1. Human drug-transporting proteins and drug-metabolizing enzymes

Advances in the field of proteomics have enabled the quantification of human drug-transporting proteins and DME levels in colonic samples. Databases such as the PRIDE repository (Perez-Riverol et al., 2022) contain a wealth of information, but many details are inaccessible without reanalyzing the data. Another major challenge in working with current colonic proteomics data is their comparability. Outcomes from published studies are not necessarily comparable, as protein identification and quantification are strongly method-dependent (Wegler et al., 2017). Accuracy has, however, significantly improved over recent years (Prasad et al., 2019). Moreover, small intestinal sampling has been prioritized over colonic sampling, as the small intestine is the main site of absorption for orally administered drugs. As of yet, there are, to our knowledge, no published studies focusing on the full native, healthy colonic ADME proteome. Published transcriptomes and proteomes collectively suggest that only few major transporters and DMEs are relatively abundant in the human colon, among them P-glycoprotein (PGp; also known as ABCB1 or MDR-1) and Breast Cancer Resistance Protein (BCRP, also known as ABCG2) (Table 2). These and other efflux pumps transport a wide range of substrates out of the epithelium. Fatty acid binding proteins are also reasonably abundant, as are Phase II DMEs, exemplified by, *e.g.*, SULT1A and UGT1A (Table 2). Phase I metabolism, conversely, appears moderate to low, exemplified by the expression levels of *e.g.* DPPs and CYP3A5 (Table 2). Most of these proteins exhibit a broad range in expression in the colonic samples characterized to date. In disease states, changes in expression patterns (Wiśniewski et al., 2015; Zhang et al., 2014) and even cell type proportions (Kelly et al., 2022) likely substantially impact the availability of colonic ADME proteins. Further proteomics studies, focusing specifically on colonic ADME proteins, are needed to ensure that both overall expression patterns and interindividual differences are properly assessed.

Table 2
Human colonic ADME proteins in healthy colon tissues and cells. 0 = not determined, + = low expression, ++ = moderate expression, +++ = high expression. For table references (given in parentheses), see Appendix.

Type	Protein	Gene	Information level (total number of patients)	Expression level
Active transport	ABCB1 (PGp, MDR1)	ABCB1	Transcriptome (n = 55–56) (17)	+/++
			Proteome (n = 4) (18)	+
	ABCC1 (MRP1)	ABCC1	Transcriptome (n = 55–56) (17)	+/++
			Proteome (n = 4) (18)	++
	ABCC2 (MRP2)	ABCC2	Transcriptome (n = 55–56) (17)	+
			Proteome (n = 30) (19)	0/+
	ABCC3 (MRP3)	ABCC3	Transcriptome (n = 55–56) (17)	+++
			Proteome (n = 4) (18)	0/+
	ABCG2 (BCRP)	ABCG2	Transcriptome (n = 55–56) (17)	+/++
			Proteome (n = 4) (18)	+
Solute carriers	ABST (IBAT)	SLC10A2	Transcriptome (n = 55–56) (17)	+
	OATP1A2	SLCO1A2	Transcriptome (n = 55–56) (17)	0
	OATP2B1	SLCO2B1	Transcriptome (n = 55–56) (17)	+/++
	OCT1	SLC22A1	Transcriptome (n = 55–56) (17)	+/++
	OCT3	SLC22A3	Transcriptome (n = 55–56) (17)	+
	PEPT1	SLC15A1	Transcriptome (n = 55–56) (17)	+
	MCT1	SLC16A1	Proteome (n = 30) (19)	+
Carrier proteins	FABP1	FABP1	Proteome (n = 30) (19)	+++
	FABP2	FABP2	Proteome (n = 30) (19)	+
	FABP3	FABP3	Proteome (n = 30) (19)	+
	FABP5	FABP5	Proteome (n = 30) (19)	++/+++
Drug-metabolizing enzymes	CYP2B6	CYP2B6	Transcriptome (n = 6) (20)	+
	CYP2C9	CYP2C9	Transcriptome (n = 6) (20)	+
	CYP2C19	CYP2C19	Transcriptome (n = 6) (20)	+
	CYP2D6	CYP2D6	Transcriptome (n = 6) (20)	+
	CYP3A4	CYP3A4	Transcriptome (n = 6) (20)	+
	CYP3A5	CYP3A5	Transcriptome (n = 6) (20)	+
			Proteome (n = 4) (18)	+
	SULT1A	SULT1A	Transcriptome (n = 6) (20)	++
			Proteome (n = 4) (18)	+++
	UGT1A	UGT1A	Transcriptome (n = 6) (20)	+/++
			Proteome (n = 4) (18)	++
			Proteome (n = 30) (19)	+/++
	UGT2B7	UGT2B7	Transcriptome (n = 6) (20)	+
			Proteome (n = 4) (18)	+++

(*continued on next page*)

Table 2 (*continued*)

Type	Protein	Gene	Information level (total number of patients)	Expression level
			Proteome ($n = 30$) (19)	+/++
	DPP3	DPP3	Proteome ($n = 30$) (19)	++
	DPP4	DPP4	Proteome ($n = 30$) (19)	+
	DPP7	DPP7	Proteome ($n = 30$) (19)	+

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3.2.2. *Microbial drug transporters and drug-metabolizing enzymes*

Current knowledge of drug accumulation and metabolism in microbes has largely been limited to the field of antibiotic research (Widya et al., 2019). Though diverse efflux pumps have been described, little is known regarding their substrate specificity beyond antibiotics (Saier et al., 2021). It is, however, notable that many drugs in current human use interact with, and in up to a fourth of the cases directly affect the growth of specific gut microbiota members (Maier et al., 2018). Indeed, microbial drug metabolism has also been exploited for decades using prodrugs like sulfasalazine (Lemmens et al., 2021b), but is still a developing research field. Microbial metabolism of selected drugs, reviewed elsewhere, has been characterized both in individual strains and whole gut microbial communities (McCoubrey et al., 2021). Likewise, the impact of microbial drug degradation on colonic absorption has been studied for selected model compounds (Tannergren et al., 2014). We are, however, still far from a complete picture of the full capabilities of individual species, let alone that of a functional microbiota network. Use of standardized whole gut microbiota models (O'Donnell et al., 2016) and studying representative strains in smaller multispecies networks (Lozano et al., 2019) will improve understanding of complex metabolic interactions.

3.2.3. *Impact of drug-transporting proteins and drug-metabolizing enzymes*

Intracellular drug accumulation is expressed as the ratio between drug concentrations in the cells and in the surrounding medium (Mateus et al., 2017). In colon tissue with modest expression of uptake transporters, relatively high expression of efflux transporters, and relatively low expression of DMEs, Kp will likely be < 1. Fatty Acid Binding Proteins (FABPs), relatively highly expressed in colonocytes, are able to bind and shuttle drugs between organelles (Chuang et al., 2008; Velkov et al., 2007). FABPs were shown not to significantly contribute to total binding in other human cell types (Treyer et al., 2018), but their impact on colonic Kp remains unclear. As so little is known about the mechanisms of drug accumulation and turnover in microbial cells, especially going beyond intentional antibiotics, much work is needed is this field. Overall, an issue to consider for both cell systems is the applicability of Kp in a colonic context. While colonic cells are generally cultured in aqueous media *in vitro*, the *in vivo* environment is characterized by a low fluid volume (Dutton et al., 2019). Improved quantitative knowledge of both human and microbial ADME proteomes is therefore especially important, allowing Kp to be scaled to accommodate the inter- and intraindividual variability in protein expression (Neuhoff et al., 2021; Wegler et al., 2021).

Summary

The colonic cellular environment is home to both epithelial and microbial cells. Respectively, within these two categories, colonocytes and goblet cells, and bacteria, dominate. While both epithelial and microbial systems can be modeled *in vitro*, more representative models will be needed to accurately determine all relevant cellular determinants of drug distribution. For the epithelium, further standardization and characterization of colonoids seems highly promising. Network-

dependent colonic bacteria will become accessible via improved *in vitro* modeling of their niche environment. Based on presently available information, impactful determinants are likely to differ between epithelial and microbial cells, due to their structural differences, as well as between healthy and disease-associated colon states. The extent to which nanosized glycan filters act as a drug sink is poorly understood overall, both in epithelial and microbial cells. Lipids, though a major sink in human cells, are likely less impactful in microbial cells, which are dominated by other types of sinks. Future characterization of these determinants of $f_{u,cell}$ will aid in understanding colonic F_{ic}. However, determinants of Kp likewise need to be considered. Presently, both drug-transporting and drug-metabolizing proteins are poorly characterized in colonic cells. Gaps such as high inter- and intraindividual variability in the epithelium and microbiota, as well as a generally poor understanding of bacterial protein-drug interactions, need to be addressed. Improved knowledge of both colonic ADME protein substrates and expression are needed not only for the assessment of F_{ic}, but also for, *e.g.*, the generation of *in-vitro-in-vivo* extrapolation scalars. Such physiologically based pharmacokinetic parameters will improve the predictability of colonic drug efficacy, closing a major research gap in the field of drug delivery.

Data availability

No *de novo*-generated data was used for the research described in the article.

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