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Porcine models of digestive disease: the future of large animal translational research

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Abstract

There is increasing interest in non-rodent translational models for the study of human disease. The pig, in particular, serves as a useful animal model for the study of pathophysiological conditions relevant to the human intestine. This review assesses currently used porcine models of gastrointestinal physiology and disease and provides a rationale for the use of these models for future translational studies. The pig has proven its utility for the study of fundamental disease conditions such as ischemia/ reperfusion injury, stress-induced intestinal dysfunction, and short bowel syndrome. Pigs have also shown great promise for the study of intestinal barrier function, surgical tissue manipulation and intervention, as well as biomaterial implantation and tissue transplantation. Advantages of pig models highlighted by these studies include the physiological similarity to human intestine as well as to mechanisms of human disease. Emerging future directions for porcine models of human disease include the fields of transgenics and stem cell biology, with exciting implications for regenerative medicine.

Introduction

Digestive disease results in more than 230,000 deaths annually in the United States, with colorectal cancer as the leading cause of mortality in adults from digestive disease (1). Animal models are imperative for translational research targeted at improving human health. Because of the limitations of directly studying human disease in a clinical setting, animal models have been used extensively to expand basic science knowledge. Rodents, particularly mice, have been commonly used animal models of disease because of their relatively low cost, ease of maintenance, and rapid reproduction rate (2, 3). This has been

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facilitated by the creation of inbred strains that represent spontaneous models of disease. Examples include TNFΔARE and SAMP1/YitFc mice strains that exhibit Crohn's disease-like ileitis as is seen in human patients (4, 5). They also make for effective models of cancer because of their high susceptibility to developing chemically induced malignancies (6). Furthermore, they are highly amenable to genetic manipulation (2, 7). The utilization of transgenic and knockout mice has provided invaluable insight into the impact of genetic mutations and specific genes on disease etiology and progression (8–10). However, murine models often lack key clinical signs or pathological changes representative of human gastrointestinal disease which are essential to improve translational studies and drug discovery (Table 1) (7, 11–19). Therefore, there is renewed interest in large animal models that more closely resemble human disease processes (20, 21) and provide a non-rodent model for drug discovery. Aside from physiological considerations, the larger size of pigs is advantageous for models requiring surgical manipulation, such as Thiry-Vella loops in which an isolated cannulated segment of intestine is studied in vivo (22), or where research involves tissue transplantation (23). Of other large animals used in biomedical research, dogs have been used extensively, particularly for the study of ischemia/ reperfusion injury. However, with increasing social pressures to limit use of dogs as experimental animals and the high mortality rate associated with some disease models, the use of dogs is declining (18, 23). However, dogs are particularly well suited and are increasingly utilized for the study of spontaneous naturally occurring diseases that also affect humans, such as cancer. In fact, clinical trials in veterinary oncology, have been used to inform drug efficacy and safety in humans (24).

The pig has a number of distinct advantages that has made this species a useful translational research animal model (Table 1). In particular, there are important anatomical and physiological similarities to human beings (19, 25). The pig has a comparably sized genome with extensive homology to humans. The pig genome has a 60% sequence homology to humans as compared to rodents with only 40% homology (26, 27). Additionally, as compared to mouse, rat, dog, cat or horse, the pig chromosomal structure is more like humans (28, 29). Pigs, like humans, are omnivores and share similar metabolic and intestinal physiological processes (19, 30, 31). For example, a comparison of the recommended daily allowances of vitamins and minerals in the human diet and the daily nutrient requirement of pigs reveal striking similarities between the two species in infancy, growth, reproduction, and lactation (19, 32). This likely contributes to their comparable mucosal barrier physiology and enteric microbiota, as well as susceptibility to select enteric pathogens (19, 33). The role of the intestinal microbiota in maintaining intestinal health has been highlighted in recent years, and disturbances in microbial composition have been associated with important human diseases such as diarrhea, neonatal necrotizing enterocolitis and obesity. Conversely, studies focusing on the relationship between the composition of the gut microbiota and disease have shown widely diverging results when comparing mice to humans (33–35). This has been addressed most recently by utilizing humanized germ-free mice transplanted with human microbiota (36–39). However, similarities in the intestinal microbial ecology between pigs and humans have made the pig a useful non-primate animal model for studies of dietary modulation of microbiota (40). Furthermore, a humanized germ-free pig model has also been established (33, 39). Under natural conditions though, both

human and porcine gut microbiota consist mainly of Firmicutes and Bacteroidetes phyla although their overall gastrointestinal microbial diversity is affected by diet, age and environmental conditions (40–42). Additionally, pharmaceutical bioavailability and nutrient digestibility in pigs closely resemble that of humans (19, 25, 43). These characteristics have led to the use of pigs for the development of pig models of a number of gastrointestinal diseases including necrotizing enterocolitis (16, 44–48), acute mesenteric ischemia (18, 49–52), short bowel syndrome (43, 53–57), AIDS-associated Cryptosporidium infection (17, 58, 59), stress-induced intestinal dysfunction (60–63), cystic fibrosis (64, 65) and familial adenomatous polyposis (14). This review will highlight strengths and limitations of pig models of intestinal ischemia/reperfusion injury, stress-induced intestinal dysfunction and short bowel syndrome. In addition, we have reviewed information that will extend the discussion on animal models in the fields of transplantation, bioengineering, and transgenics.

Comparative gastrointestinal anatomy: Similarities and differences between humans and pigs

Pigs have significant anatomical and physiological similarity with human beings, with some key comparisons noted in Table 2 and Figure 1. The structure of the small intestine is very similar in humans and pigs, including macroscopic features such as the ratio of intestinal length per kilogram bodyweight (Table 2) (19). Other gross similarities include the presence of sacculations and taenia (bands of longitudinal muscle) extending along the majority of the colonic length in both human and porcine colons (30, 66). These anatomical similarities contribute to the comparable transit time and analogous digestive and absorptive processes reported for these species (67). Shared microscopic features also exist, including the structure of the villi and the types of cells that constitute the intestinal epithelium (Figure 1). For example, the villous projections within the small intestine of both species are finger shaped, whereas in rats they are flat leaf-like structures (30). Additionally, the cell lineages that constitute the intestinal epithelium, their phenotypic appearance, and their expression of unique protein biomarkers used for cellular identification are similar in pigs and humans (68). These include the ultrastructural appearance of stem, goblet, and enteroendocrine cells as well as absorptive enterocytes (68). A comparative analysis of sub-cellular features of porcine intestinal epithelial cells located within the crypt base, using transmission electron microscopy, revealed multiple irregularly shaped, small, columnar cells with basally located nuclei and scarce cytoplasm (68). This morphologic appearance is consistent with the description of crypt base columnar stem cells in humans (68, 69). Taken together, these features contribute to fundamental digestive processes such as mucosal transport and motility that are similar between pigs and humans (19). Additionally, pigs, like humans, are true omnivores, whereas other potential mammalian models such as dogs, cats, ruminants, rabbits and rodents have evolutionarily developed alternative digestive strategies (30, 70). Furthermore, pigs and humans have similar neonatal gut development and gastrointestinal immunologic responses to insult (30, 70, 71). As far as differences in gastrointestinal tract anatomy, the porcine cecum is relatively large and clearly delineated as compared to the human cecum, and the porcine colon is orientated in a spiral fashion (Figure 1) (30). Pigs also lack an appendix (30, 70). Other important considerations when using the pig as an

animal model to study human development and disease is their rapid growth rate, their size once fully mature and differences in gut-associated lymphoid tissue (40, 72). For example, differences have been identified in the development, distribution, cellular composition and number of Peyer's patches between pigs and humans. In general, Peyer's patches are considered important in the gut to help discriminate between pathogenic and commensal bacteria however the significance of the differences between species remains unclear, especially in light of using the pig to model human intestine (72–74). Despite these differences, both pigs and humans are colon fermenters, unlike rodents that are cecal fermenters, and have similar colonic microbiota composition (32, 33).

On a cellular level, there is controversy within the literature of the presence or absence of the Paneth cell within the pig (75–77); a fully differentiated cell type that secretes antibacterial substances and has a proposed role in supporting the adjacent crypt base columnar cells within the small intestinal crypt base of other species, including humans (78–80). However, based on recent work, a cell sharing ultrastructural qualities attributed to the Paneth cell, including large size, elongated flattened nucleus and located adjacent to the crypt base columnar cells has been identified within porcine small intestine (68). These cells lack certain morphological features historically used to identify Paneth cells such as electron dense apically situated granules. Furthermore, no commercially available antibodies have thus far demonstrated cross reactivity to lysozyme, a well-accepted and commonly used biomarker for Paneth cells (68). However, further research is required to conclusively prove whether the cells that reside next to the crypt base columnar cells in pigs share the same functional role as the Paneth cell in other species. Never the less these differences appear to have relatively little effect on overall physiological function, which is critical when selecting an animal model for human digestive disease. In addition, there are important similarities between other cell populations thought to be critical to maintenance of the stem cell niche. For example, antibodies raised against human-derived proteins utilized as specific biomarkers for the identification of stem, progenitor, absorptive enterocyte, enteroendocrine and goblet cells cross react with porcine intestinal tissue, and demonstrate the same expression pattern observed in humans (68). These include antibodies against both SOX9 and HOPX that identify stem and progenitor cells. It should be noted, that antibody based tests are limited by available functional antibodies and none have specifically identified the crypt based columnar population. However, it has been shown that pigs do express crypt base columnar cell gene biomarkers (68). Work is needed to further develop the pig as a model to study intestinal stem cells however, considering what is established, the pig serves as a valuable model for studies of human intestinal injury, repair and efficacy of novel therapeutics (Table 3).

Psychological stress-induced intestinal dysfunction

Stress plays a central role in the onset and exacerbation of clinical symptoms in several gastrointestinal diseases of humans. Progress in the field of stress-related gastrointestinal disease has been hampered by the lack of relevant animal models of stress that recapitulate the chronic and complex biology of stress and the clinically relevant outcomes (e.g., diarrhea and weight loss) that are observed in humans. Much of the work in this field has utilized rodent models of short term/acute stress (81, 82). These models have yielded

important insight into the mechanisms by which stress initiates gastrointestinal disease; however, there are obvious developmental and biological differences between rodents and humans that limit their translational implications. Porcine models are ideal for studying stress-related gastrointestinal disease because: (1) compared with rodents, the porcine gastrointestinal tract is most similar to humans with regard to development, anatomy, and function (83); (2) pigs possess a highly developed central and peripheral nervous system in order to perceive and integrate complex stress signaling processes in the brain and peripheral nervous systems (84); and (3) pigs, similar to humans, exhibit pathophysiological hallmarks of disease (intestinal inflammation) and relevant clinical signs (e.g. diarrhea and reduced weight gain) when subjected to stress.

Altered function of the enteric nervous system (ENS) plays a central, pathophysiological role in stress-related gastrointestinal diseases as it modulates stress-induced changes in motility, inflammation, intestinal permeability, and visceral hypersensitivity; therefore, the ENS is currently a therapeutic target. The porcine ENS has been shown to have similarities to human ENS that may offer advantages over commonly used rodent models (84). For example, intrinsic primary afferents in the human and porcine enteric nervous systems differ significant from rodents and other small laboratory animals in their distribution electrophysiological behavior, and synaptic properties (84, 85). These interspecies differences are important when considering animal models of human disease because it is imperative that therapeutic treatments for stress associated intestinal disorders such as IBS are based on results from animals with extensive similarity in their gastrointestinal tracts (84).

A novel porcine model of stress-induced gastrointestinal dysfunction that exhibits remarkable similarity to the pathophysiology of stress-related gastrointestinal disease in humans is the porcine early weaning stress model. In the porcine early weaning stress model, pigs are weaned from the sow at an early age (prior to 21 days of age), as compared to the natural weaning process which occurs gradually over months. The early weaning model has been valuable in studying the mechanisms of early life stress-induced gastrointestinal disease (60–63) while at the same time understanding the impact of stressor on gastrointestinal health in veterinary medicine. Early weaning stress is a common stress in pig production and involves an initial early life stress event followed by consistent, heterotypic stressors associated with changes in management and social conditions until they have reached market weight. This condition models aspects of heterotypic chronic stress in humans. Early weaning in pigs induces a stress response remarkably similar to humans and is characterized by elevated serum and intestinal corticotropin releasing factor (CRF) and subsequent modulation of intestinal mast cells that has been shown to play a critical role in intestinal permeability and diarrhea in pigs and humans (60, 61, 63, 86). In addition to the CRF-mast cell axis, other stress-induced neurochemicals may be involved in the intestinal pathophysiology associated with stress. For example, it has been shown that adrenocorticotropic hormone (ACTH) and norepinephrine (NE) increased the attachment of enterohemorrhagic Escherichia coli O157:H7 (EHEC) to isolate ex vivo porcine intestinal mucosa (87, 88).

Cholinergic and noradrenergic stimulation in isolated ex vivo preparations of porcine colonic mucosa was also shown to stimulate secretory IgA from the porcine intestine which in turn could modulate mucosal immunity during the stress response (89). Together, these findings suggest that mediators released during the stress response, such as NE and ACTH, may directly enhance the binding of bacteria, stimulate IgA secretion potentially modulating the intestinal stress response.

Ischemia/ reperfusion injury

Intestinal ischemic injury occurs when the blood supply to a segment of intestine is compromised. Paradoxically, tissue damage may continue when blood flow is reestablished and this is referred to as reperfusion injury. Injury attributed to ischemia/reperfusion injury is associated with many disease states including: intestinal volvulus (90), acute mesenteric ischemia (91), intestinal transplantation (92) and systemic disease that lowers tissue perfusion such as hemorrhagic shock or cardiopulmonary disease (93, 94). In general, an excess of 50% of patients suffering from intestinal ischemia/reperfusion succumb to sepsis associated with a breakdown in intestinal barrier function (95). In addition, ischemia is thought to contribute to necrotizing enterocolitis (NEC), the most common life-threatening gastrointestinal emergency in neonatal patients (96–98). Ultimately, mortality depends on the duration, location, and length of bowel affected by impaired perfusion. The type and degree of injury to the intestinal mucosa resulting from a pathologic decrease in intestinal blood flow is influenced by the microvascular architecture within the villi. Experimental models of ischemia have shown that the morphological signs of injury begin at the villus tip and progress toward the base of the villus (15). The villus circulation comprises blood entering the villus via a centrally-located arteriole, which arborizes at the tip of the villus and converges into venules located beside the arteriole and on the periphery of the villus (99). This sets up a counter-current exchange whereby oxygen diffuses from the arterioles toward low oxygen tension venous blood flowing in the opposite direction within the venules. Diffusion is facilitated by the relatively low rate of blood flow within villi. This in turn makes the tip of the villous relatively hypoxic even under normal conditions, which is exacerbated when blood flow is reduced or obstructed. However, the degree to which ischemia promotes mucosal injury is dependent upon the anatomy of the villous microvasculature, which affects the efficiency of counter-current exchange. Importantly, this microvascular architecture varies between species. The pig and human villous microvascular architecture are very similar, arborizing at the tip of the villous into a fountain-like pattern and converging into one or two venules located beside the arteriole (99, 100). The vascular architecture in rodents varies from that found in humans and is thought to influence the functional differences that have been appreciated between the mucosa of these species and may contribute to differences in mucosal susceptibility or response to injury (101–104). The specific microscopic architectural features of pigs and humans that are shared including villus structure and vascular anatomy likely contribute to interspecies similarities in ischemic damage (18, 101). Additionally, since many forms of intestinal ischemic injury involve decreased tissue perfusion and the villi are the first and most dramatically damaged in ischemic injury, the pig may more accurately model this disease process in humans (15, 105).

In clinical cases, the degree of damage varies according to the type of ischemic injury, and it is therefore not possible to fully understand all of the features of ischemia/ reperfusion injury with a single model. In light of this, porcine studies elucidating mechanisms of ischemia/ reperfusion injury relevant to human disease have assessed mesenteric ischemia, volvulus, and hemorrhagic shock to understand the relative contribution of ischemia and reperfusion to mucosal injury (18, 22, 23, 49–52, 106–108). Mesenteric ischemia was found to cause the most severe time-dependent injury, which was marginally exacerbated by reperfusion, and only at select time points (2-hours of ischemia, 1-hour reperfusion) (15). Alternatively, low-flow ischemia induced by hemorrhagic shock in pigs caused minimal ischemic injury, with no evidence of reperfusion injury (15). This is in marked contrast to rodent and feline studies exploring a range of low-flow states, all of which induced minimal ischemic injury, but were accompanied by marked reperfusion injury (109–113).

A great deal of the research on ischemia/ reperfusion injury has focused on the reperfusion phase, with the hope that basic findings would translate to the clinical ability of blocking injury by treating prior to reperfusion. Mechanisms of reperfusion injury have largely been documented in rodent and feline models, with the seminal discovery of xanthine oxidase as a central enzyme in the development of reactive oxygen metabolites (ROM) (15, 109, 110, 114–116). Studies in these laboratory animals have shown that small intestinal ischemia is followed by a robust reperfusion injury as a result of mucosal xanthine oxidase-induced oxidant release. However, there is doubt as to the clinical relevance of these findings in humans, which lack mucosal xanthine oxidase at birth, and intestinal enzyme expression remains relatively low throughout development to adulthood (15, 117). This pattern of xanthine oxidase expression is very similar in pigs, whereas rodents maintain levels 4–5 fold higher (15). Therefore information gleaned from rodent and feline models that attribute reperfusion injury to activation of xanthine oxidase may not be readily extrapolated to humans or may be relevant only to particular types of injury (10, 109, 111, 118–120). Another critical element shown to induce and perpetuate reperfusion injury is the neutrophil. Neutrophils are activated by chemoattractants induced by reactive oxygen metabolites, after which they greatly amplify reperfusion injury (121). The key neutrophil population involved in reperfusion injury in rodents is the resident mucosal population, as opposed to neutrophils recruited from the circulation (122). However, pigs have a relatively small population of mucosal neutrophils, as do humans, in contrast to the large population of resident mucosal neutrophils in rodents and cats (15). Collectively, these findings may explain the relative lack of reperfusion injury in the pig under a variety of ischemic conditions, including mesenteric ischemia, volvulus, and shock. These considerations are relevant to effective translation of basic science research to the treatment of human ischemia/ reperfusion injury (104, 123, 124).

The physical size of the pig as a model of human disease also enhances the feasibility of creating surgical models that recreate clinically relevant disease, including acute mesenteric ischemia (AMI) (18, 49, 108). Approximating the human size is also crucial in the development and testing of imaging modalities for early diagnosis of AMI (91, 125). This is likely why research focused on improving early diagnosis of AMI commonly utilizes porcine models to evaluate the efficacy of imaging modalities such as radiography, angiography, computed tomography and magnetic resonance imaging (50–52, 106, 126).

Mucosal repair

The slow progress in translating basic science findings on reperfusion injury to clinical trials has been reviewed elsewhere (123, 124). More information on mechanisms of mucosal repair is critical. Three important stages of intestinal epithelial repair have been identified. First, the immediate post injury or acute stage of repair is when barrier function is re-established by villous contraction and cellular restitution. The process of epithelial restitution has been re-examined in porcine models of ischemic injury, and found to include a novel component that had received little attention in cell models: tight junction repair (127–129). Specifically, initial studies on post-ischemic epithelial repair in pigs revealed a lack of barrier function recovery until tight junctions within restituting epithelium had closed (128). Thus, the term restitution now integrates the concepts of villus contraction, epithelial crawling, and tight junction re-organization. This proliferation independent process ensures that the basement membrane is covered, epithelial continuity is established and inter-cellular tight junctions are re-assembled (127, 130). During the subsequent or sub-acute period, cellular proliferation, differentiation and the onset of inflammation predominate (22). Finally, during the chronic stage of mucosal repair the normal mucosal architecture is re-established, the small intestinal villi for example, in addition to varying degrees of fibrosis that depend on the initial degree of injury and inflammation (131). Each of these processes has been dissected in depth, particularly the latter which continues to be a strong thrust of porcine studies (132–134). In terms of translation of such studies, the potential for pharmacologically hastening tight junction repair has been demonstrated in porcine post-ischemic bowel and awaits human trials (135).

Evaluation of mucosal repair following ischemic injury in pigs has also revealed a different time course of neutrophil infiltration as compared to murine and feline studies. In particular, ischemia in rodents and cats showed peak infiltration of neutrophils within 1-hour of reperfusion injury (116, 136, 137), whereas in porcine studies, peak neutrophil infiltration occurred 6-hours following ischemic injury (121). The importance of this finding was that rather than exacerbating injury, this delayed neutrophil infiltration may hamper restitution, particularly tight junction repair, as neutrophils coursed through the paracellular spaces of restituting epithelium. Of translational significance, inhibiting neutrophil adhesion, or treating with superoxide dismutase could restore optimal repair (121). Porcine studies have also shown that neutrophils induce physical damage to the epithelial tight junctions as they transmigrate across ischemic-injured mucosa (121). Other porcine studies have focused on understanding mechanisms of tight junction repair following ischemic injury, including clinically relevant pharmacological interventions (22, 135, 138, 139).

Necrotizing enterocolitis

NEC is an abdominal emergency that afflicts approximately 10% of infants born with low birth weight, and has a mortality rate of approximately 30% (16, 98). Severe, irreversible damage to the intestine often necessitates extensive surgical resection with resultant short bowel syndrome (SBS). This syndrome is associated with debilitating malnutrition, diarrhea, abdominal pain and fatigue (98, 140). The piglet is the only animal model that develops NEC under the same conditions that predispose human infants, namely prematurity,

bacterial colonization and enteral feeding (16, 45, 141). Pigs also demonstrate pathognomonic signs of human disease including abdominal distension, food intolerance and regurgitation as well as mucosal necrosis in the distal small intestine and colon and pneumatosis intestinalis (40, 45, 142, 143). One of the most important features of NEC shared by neonates and piglet models is that disease is only seen in premature individuals (48). Importantly, 'total parenteral nutrition' (TPN), which is associated with onset of NEC in preterm infants, can be administered to piglets. Piglets not only share predisposing factors and clinical signs of NEC but also the histologic indicators of disease. This has led to extensive work utilizing the pre-term piglet model for treatment and drug discovery to improve the care of affected infants (44–47, 141, 143–146). For example, the severity and degree of mucosal atrophy were reduced in a piglet model when probiotics consisting of *Bifidobacterium animalis* and four *Lactobacillus* species were administered post parturition (47). Notably, the positive findings in porcine studies have been applied to human subjects and have confirmed the decreased incidence of NEC resulting from probiotic therapy (40, 147, 148).

Short bowel syndrome

Although the preterm piglet is an ideal model of SBS in NEC patients, variations between infant, juvenile and adult growth rates as well as developmental physiology preclude extrapolation of knowledge based on neonatal models of SBS to older patients. Here again, pigs provide a translational advantage because developmental and age-related changes in the gut are similar between pigs and humans (43, 149–151). Short bowel syndrome as a result of extensive resection of ischemic-injured or Crohn's disease-affected bowel is the most common cause of intestinal failure in adults (140). Following extensive intestinal resection, the mucosal surface area is decreased, impairing nutrient and water absorption. This may result in diarrhea, fluid and electrolyte abnormalities, and malabsorption and weight loss (140). To offset SBS, the intestinal remnant undergoes an adaptive response that includes villus hyperplasia, increased crypt depth, and increased brush border enzyme activity that results in increased mucosal surface area with improved fluid and nutrient absorption. Although, morphological and functional changes characteristic of intestinal adaptation have been reported in rodents, the pig provides a more suitable model to study specific nutritional requirements critical to the treatment of SBS patients (31, 32, 43, 56, 57, 152–156). Total parenteral nutrition is currently the treatment of choice in humans and as was previously noted, can be administered to pigs (140). However, treatment with TPN is fraught with complications in SBS patients. These include venous thrombosis, infection and organ failure as well as sleep disruption, anxiety and depression (140). These clinical obstacles have driven extensive research into ways of minimizing TPN use by identifying effective mitogenic agents that hasten intestinal adaptation and improve the absorptive capacity of the remaining intestine. Murine models have successfully demonstrated the mitogenic effects of Glucagon-like peptide 2 (GLP-2) therapy; however, the FDA required the use of non-rodent models for pharmaceutical testing prior to human clinical trials. Porcine studies effectively demonstrated the rapid intestinal growth and maturation, increased intestinal brush border enzyme expression, decreased apoptosis and proteolysis, increased nutrient absorption and intestinal blood flow following treatment with GLP-2 (145, 157–162). GLP-2 has now

emerged as a potential therapeutic option for some patients and is currently used in humans (163–165). Further work has focused on the appropriate time frame for administration of therapies aimed at enhancing the adaptive response (55). For example, a porcine model was utilized to specifically identify changes in the absolute numbers of epithelial cells within distinct mucosal populations and the time course of those changes in remnant bowel following extensive resection to give insight into potential targeted therapies (55). That study found that despite an early increase in proliferative and enterocyte cell number, following bowel resection, the cells were immature and incapable of normal absorptive function. It was therefore concluded that therapeutic interventions should be aimed at increasing cellular differentiation into mature cell types, not just general cellular proliferation to hasten return of normal absorptive function (55).

Transplantation studies

Ultimately, when extensive and irreversible injury to the intestine occurs and when TPN therapy fails to effectively maintain SBS patients, intestinal transplantation is indicated (140). Unfortunately, there is an unacceptably high mortality following intestinal transplantation. For example, death occurs in 40% of patients within five years of receiving an allograft (166). Acute rejection, chronic allograft dysfunction and ischemia/ reperfusion injury within the transplanted bowel are the most severe and common complications associated with bowel transplantation (23, 92). The limited options for replacing resected intestine make allotransplantation, xenotransplantation and tissue engineering research integral to improving available therapies for SBS patients. The pig represents an ideal model for each of these types of studies because of their shared size and specific physiologic, immunologic and organ developmental similarities with humans that translate into answering questions of practicality and generalized efficacy (23, 167–170). For instance, the current reference standard for detection of acute cellular rejection of allografts is a histological grading system (171) similar to the grading system used to identify allograft rejection in pigs used in transplantation research (23). The grading scale ranges from mild rejection, as determined by the presence of greater than 6 apoptotic cells out of 10 crypts and a mild to moderate inflammatory infiltrate of predominantly mononuclear cells, to severe rejection characterized by crypt destruction, mucosal erosions and severe inflammatory infiltration (23).

Although rodent models of allotransplantation have contributed to improvements within the field, the increased gross size of the pig permits investigation of alternative surgical therapies that mimic those possible in humans, combined with low mortality in experimental animals as compared to rodents (23, 172, 173). Examples of porcine models of transplantations include: orthotopic (graft in gastrointestinal continuity), heterotopic (graft not in gastrointestinal continuity), segmental (Thiry-Vella loop), whole small bowel or combined small bowel with colon (23, 170). Therefore, porcine experimental work directly facilitates the development of procedures for use in clinical patients (23, 170, 173).

Despite improvements in the success of alloengraftment surgery in recent years, particularly in specialized centers, recipients must cope with lifelong immunosuppressive therapies and the co-morbidities and mortality associated with this therapy (140, 169). The limitation of

rodent models for these translational studies is associated with the major immunological differences that exist between species. In recent years, significant advances have been made in the development of 'humanized' mice to study human biological processes. However, these developments do not preclude the need for a large, more biologically complex system, to verify findings (38). Although there are immunological differences between humans and pigs, specific similarities and recent advances in the development of genetically modified animals has made the pig the most likely potential source for xenografts for humans (25, 168, 174). The demand for human organ donations significantly overwhelms the supply with over 100,000 recipient candidates awaiting organ transplants in the U.S. (175). The first transgenic pig was generated in 1985 and the first swine model derived specifically for the betterment of human health was in the field of xenotransplantation (175, 176). Pigs are considered the preferred donor species because their organ size is compatible with humans and because using non-human primate species poses difficult ethical concerns and infectious diseases (168, 177, 178). The development of genetically modified pigs expressing human complement pathway regulatory proteins (hCPRPs) or that have the α1,3-galactosyltransferase gene "knocked-out" (GalT-KO), has overcome the initial barriers posed by acute graft rejection (177–179). With these genetic modifications to inhibit recipient immunologic responses, porcine to non-human primate heart and kidney transplants have been successful (180).

Bioengineering offers an alternative source of tissue for intestinal replacement and avoids the complications associated with ischemia/ reperfusion injury of the donor tissue and life-long immunosuppressive therapy for the transplant recipient. Creating bioengineered tissues is an exciting prospect for regenerative medicine in the future, but advancements have been hindered by the anatomic and physiologic complexity of the intestine. Compared to other areas of intestinal research, studies on intestinal tissue engineering to replace segments of native intestine are limited (174, 181, 182). A porcine model was successfully used to generate tissue-engineered small intestine from autologous tissue, thereby re-creating conditions required for successful clinical use (174). Organoid units (isolated intestinal crypt cultures with epithelial stem cells and intact surrounding mesenchymal elements) were derived from resected small intestine, placed on biodegradable polyglycolic acid tubes, and implanted into the omentum or mesentery of the autologous animal. Intestinal tissue composed of all anatomic layers, including mucosa adjacent to an innervated muscularis, was shown to have developed (174). The far-reaching therapeutic potential of this research could serve as the basis for tissue replacement for a multitude of diseases. Ultimately, large animals and particularly the pig are biologically complex systems that serve a critical role in addressing the safety of a therapeutic regimen and verifying mechanistic findings from rodent models.

Transgenic pigs

The impetus for advancement in the field of transgenic pig development has been due to limitations in rodent models when their small size, short life span or inadequate representation of a disease phenotype prevents successful translational research (12). The majority of transgenic pig work has been in the field of xenotransplantation, as previously discussed. However, in recent years there have been significant improvements in the

technology used to create genetically modified pigs to model human diseases (11, 12, 175). Comprehensive tables listing the available genetically modified porcine models for use in biomedicine and of human disease can be found in Whyte et. al. and Fan et. al. (11, 175). One of the best examples with regard to intestinal disease is the development of the porcine model of cystic fibrosis (CF) (64). Mouse models lack typical disease manifestations, including the intestinal obstruction phenotype that is observed in human patients (175, 180). Transgenically modified $CFTR^{-/-}$ and $CFTR^{\Delta F508}$ pigs have proven superior by displaying the same clinical signs as seen in humans, including intestinal, bile duct, and pancreatic duct obstructions, as well as liver lesions, low levels of IGF-1 and the hallmark phenotype of lung disease (65, 180).

Previously, CF was the only intestinal disease with a representative porcine model (64, 183). However, gene-targeted pigs with mutations in the *adenomatous polyposis coli* (*APC*) gene have recently been established (14). Colorectal cancer is one of the most frequent causes of cancer worldwide and is the leading cause of gastrointestinal related mortality (1, 184). For this reason, much emphasis has been placed on the study of familial adenomatous polyposis (FAP) in humans. Murine *Apc* mutants have been used in this research but have significant limitations including differing location and histologic appearance of lesions as compared to humans (14, 185). In particular, in humans, colonic adenomas develop during childhood, whereas gastric polyps and duodenal adenomas occur during adulthood (14). In contrast, murine models develop polyps in the duodenum and small bowel, and these polyps lack dysplastic cells in the superficial mucosa (14). The porcine model of FAP, on the other hand, shares the anatomical location and histologic appearance of human disease (14). With regard to the diagnosis and treatment of FAP, the porcine model illustrates the advantage of large animals to facilitate advances in pharmaceutical, endoscopic, and surgical interventions. The completed pig genome project will provide the platform for further transgenic work to create pertinent pig models of human disease, particularly where rodent models fail to recapitulate clinical signs of disease (180).

Future promise for translational research

The need for large animal models to improve translational research is well accepted (20, 21). The limited availability of human-derived tissues requires that information gained from animal models is extrapolated to humans. The fact that mice and other rodents are the most commonly used preclinical models has been implicated in the "*pipeline problem*" (20, 186). One of the reasons for this problem is that rodent injury models fail to recapitulate human disease. For instance, murine injury models used in the study of intestinal epithelial stem cells (IESCs) have utilized chemical or mechanically induced mucosal injury or whole body irradiation to create damage (172, 187–190). None of these are common sources of mucosal injury in human clinical cases. Therefore, a great deal of additional research is needed before stem cell therapy can become a clinical reality. For example, a study examining the engraftment potential of IESCs following mucosal injury in Thiry-Vella loops was hampered by high mortality rates and the authors indicated the need for a large animal model in which Thiry-Vella loops are well tolerated (172). Until recently, a large animal model to study intestinal stem cell biology remained elusive. Currently, the cross reactivity of commercially available antibodies to identify specific cell types in pig intestinal tissue has

been demonstrated (14, 55, 59, 68, 174, 191). Validation of tools for histologic, protein and mRNA based analysis of porcine IESCs have also been completed (68). Additionally, this study describes a method for successful long-term culture of porcine crypts into fully differentiated enteroids (isolated intestinal crypt cultures with epithelial stem cells without intact surrounding mesenchymal elements). Compared to the availability of reagents and tools for the study of rodent tissue, those for pig remains limited. However, recent work provides a platform for translational research, including for stem cell engraftment studies that are currently limited by the small size and high mortality rates of rodents (172). The pig as a model to study IESCs in disease affords the opportunity to bridge the gap between lab bench discovery and bedside application.

Abbreviations

SOX9	Sex Determining Region Y-box 9
HOPX	Homeodomain-only protein X
ENS	Enteric Nervous System
IBS	Irritable Bowel Syndrome
CRF	Corticotropin Releasing Factor
ACTH	Adrenocorticotrophic hormone
NE	Norepinephrine
NEC	Neonatal necrotizing enterocolitis
ROM	Reactive Oxygen Metabolites
AMI	Acute Mesenteric Ischemia
SBS	Short Bowel Syndrome
TPN	Total Parenteral Nutrition
GLP-2	Glucagon-like Peptide 2
hCPRPs	Human Complement Pathway Regulatory Proteins
Gal T-KO	α1,3-galactosyl transferase gene knock-out
CF	Cystic Fibrosis
FAP	Familial Adenomatous Polyposis
IESCs	Intestinal Epithelial Stem Cells

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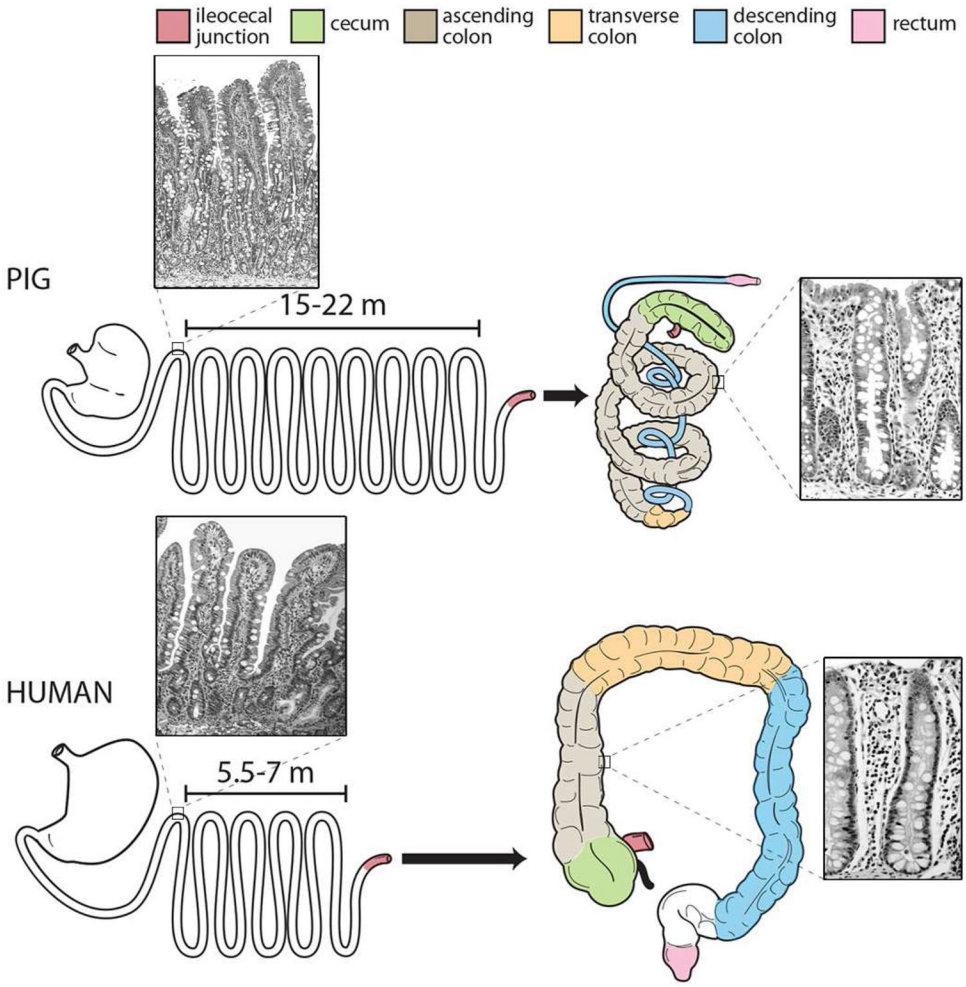

Figure 1. Comparative Gastrointestinal Anatomy

The porcine gastrointestinal anatomy (top) shares many similarities and some distinct differences to that of humans (bottom). The porcine intestinal length is greater than humans but the ratio of total length per kilogram of bodyweight for both pigs and humans is similar. The comparable portions of porcine and human colon are demonstrated with color. Images of histologic sections of porcine and human duodenum and colon are included for comparison.

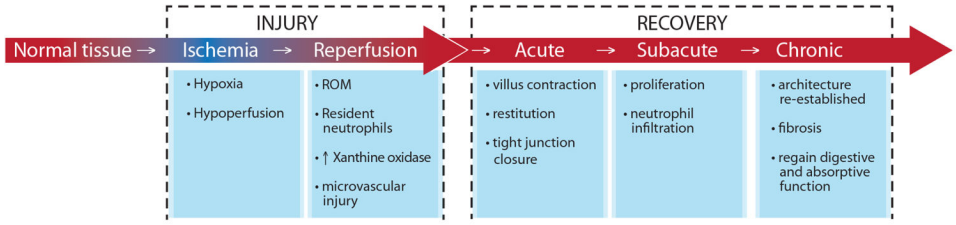

Figure 2. Ischemia Reperfusion Injury and Recovery

Intestinal ischemic injury occurs when the blood supply to a segment of intestine is compromised. Paradoxically, tissue damage may continue when blood flow is reestablished, a process call reperfusion injury. Following the initial injury induced by ischemia and reperfusion there are three important stages of intestinal epithelial repair. There is an immediate post injury or acute repair, a subsequent sub-acute period when cell proliferation occurs and a final, chronic stage of mucosal repair when normal architecture is re-established. ROM = Reactive Oxygen Metabolites

Table 1

Comparison of Porcine and Rodent Models of Digestive Disease

	Advantages	Disadvantages
Porcine	• Models exhibit pathophysiology and clinical signs of disease • Approximate human size • Xenotransplantation potential • TPN administration possible • Share nutritional requirements • Fulfill FDA requirement for pharmaceutical testing • Comparable microbiome • Possess highly developed central and peripheral nervous system • Genome is only ~7% smaller than humans	• Cost • More difficult to manage • Special housing facilities • Few transgenic models available • Time consuming
Rodent	• Low cost • Ease of maintenance • Ease of genetic manipulation • Rapid reproduction rate • Number of available and well established models	• Pathophysiological differences • Absence of clinical signs of human disease in some models • Technically difficult to create surgery models due to size • Mortality rate • Genome is ~14% smaller than humans

Table 2

Anatomical Comparison between Pigs and Humans (Adapted from Patterson et al. (19)

	Human	Pig
Average birth weight (kg)	3.4	1.4
Average mature weight (kg)	60–100	200–300
Water composition at birth (%)	82	82
Water composition as adult (%)	69	72
Average lifespan (years)	80	20
Small Intestinal length as adult (m)	5.5–7	15–22
Large Intestinal length as adult (m)	1.5	4–6
Length of intestine per kilogram bodyweight (ratio)	~0.1	~0.1
Small Intestinal weight as adult (g)	1040	2310
Large Intestinal weight as adult (g)	590	1970

Table 3

Direct Clinical Application of Porcine Models

Clinical Impact	Research Support Using Porcine Models
• **Understanding role of early life stress on onset and exacerbation of clinical symptoms of human gastrointestinal diseases**	Early life stress induces a marked elevation of serum and intestinal corticotropin releasing factor (61) that plays a critical role in intestinal permeability and diarrhea Administration of CRF receptor antagonists and mast cell stabilizers to pigs prevented stress-induced increases in intestinal permeability (60, 61, 63, 86)
• **Treatment of reperfusion Injury**	Activation of xanthine oxidase plays less important role than initially postulated in inciting injury Injury is mediated by neutrophil release of oxygen metabolites and transmigration through epithelial tight junctions creating physical damage (121)
• **Treatment of NEC patients**	Oral probiotics reduce the incidence and severity of NEC in very low birth weight infants (148)
• **Dietary considerations following massive bowel resection**	GLP-2 hastens intestinal adaptation; FDA drug approval (43, 56, 57, 163–165)
• **Successful intestinal transplantation**	Simultaneous liver transplant does not decrease the risk of bowel graft rejection and is no longer recommended in patients with SBS with normal liver function (23)
• **Cause and treatment of gastrointestinal dysfunction associated with cystic fibrosis**	Pig model of CF accurately displays clinical manifestations of human disease permitting study of pathogenesis of intestinal obstruction (11, 12, 175, 192)
• **Diagnosis of Familial Adenomatous Polyposis**	Porcine transgenic model accurately represents disease manifestation, including location and histologic appearance of lesions (14)