

Swine Model

Of the large animal species used for biomedical research, swine is easily the most popular. There are hundreds of breeds available worldwide, some of which are classified as miniature swine and commonly known as minipigs. Swine reach sexual maturity early, breed year-round, and deliver as many as 10 to 12 piglets in a single litter. Swine are large enough and robust enough to tolerate complex experimental protocols over an extended period of time that require multiple interventions, repeated tissue or fluid sampling, and imaging using technologies standard to hospitals. There is broad availability of a range of established cell lines derived from a variety of swine tissues and the offering of swine-specific reagents is expanding. Preliminary data from the swine genome project confirms the phylogenetic status of swine as closer to humans than rodent species, lending further weight to the selection of swine as a model for biomedical research. Further, swine genomics and proteomics are more advanced than nearly every other large animal model. [1-3]

It is of course most important, when selecting a model for biomedical research, that the model closely approximate the human condition under study. In this case as well, swine has enormous advantages over small mammal or invertebrate model systems. Swine are very similar to humans in various aspects of their anatomy and physiology, diet and metabolism, and histopathology and pharmacokinetics. [1-3] Over the years, swine have been used to model so many different aspects of human physiology and pathology that it would be impossible to mention them all here. Thus, this summary should be taken as neither comprehensive nor definitive, rather as an overview of some of the more prominent fields in which swine serve as important models.

Cardiovascular Disease

The swine heart is particularly suited to the study of human cardiovascular disease as its gross anatomy is very similar and its coronary microvasculature is nearly identical to that of humans – blood supply to the heart is right-side dominant and lacking in pre-existing collateral vessels. Physiologically, swine resting heart rates and left ventricular (LV) pressures are comparable to humans. Experimentally, swine offer advantages over smaller animal models of cardiovascular disease simply because of their larger size. For example, swine can tolerate multiple biopsies from the same tissues, intracoronary drug delivery, and implantation of microdialysis probes. [4,5] Swine have been used in the development and testing of intravascular stents, aneurysm surgery, valve replacement, cardiac transplant, and cardiac assist devices. [1] They have also served as important pre-clinical models for testing of new pharmacological agents and more recently stem cell therapy and gene therapy. Stem cells administered to swine post-myocardial infarction (MI) have been shown to decrease MI expansion and improve LV ejection fraction and gene therapy results using a swine model of heart failure (HF) have proved so effective that clinical trials have already begun. [4]

Swine also function as important models of MI, ischemia reperfusion (IR) injury, HF, and dilated cardiomyopathy (DCM). One of the main advantages of the use of swine as a model of cardiovascular disease is the ease with which MI can be produced in predictable sizes and locations analogous to MI in humans. Thus, swine are commonly used in pre-clinical studies investigating new strategies for limiting IR injury, infarct expansion, and LV remodeling. Post-MI, swine are positively affected by all of the cardioprotective strategies currently available for humans including hibernation and ischemic pre- and post-conditioning. Swine HF closely mimics that of humans in many respects as LV functions is depressed (*i.e.*, myocyte contractility is impaired and ejection fraction is reduced) and the

neurohormonal axis is activated. Additionally, DCM brought on by pacing-induced tachycardia in swine approximates the human DCM phenotype as it results in LV dilation, pump dysfunction, and neurohormonal changes similar to clinical observations. [4,5]

Wound Healing & Melanoma

Swine skin is remarkably similar to human skin and as such has become a standard model for reconstructive and plastic surgery and wound healing. Anatomically, swine skin is largely hairless, has a thick epidermal layer, a fixed subcutaneous layer, and a human-like pattern of cutaneous blood supply. Physiologically, swine skin responds as human skin does to various growth factors and cytokines and displays a wound healing process, similar to that documented in humans, in which re-epithelialization rather than contraction occurs. Swine skin has been used in the development of skin surgical techniques, laser therapy, and burn care and the study of dermatological conditions including vitiligo, dry skin, aseptic necrosis, hypertrophic scarring, melanoma, and others. [1,6]

Burn injury results in hypertrophic scarring (HS), which produces permanent hard, red, raised scars that are painful, disfiguring, and often debilitating. Despite decades of research, there is still very little known about the pathophysiology of HS and no treatments are available. Experimental models have been attempted using mice, rats, rabbits, dogs, and cats, but have been abandoned due to their failure to mimic human HS. By contrast, swine appear to produce scarring most similar to human HS as measured by clinical and histological appearance, biomarker presence, and nerve fiber, mast cell, and myofibroblast populations. The swine model of human HS is not perfect, but currently presents the best overall approximate for studies of burn wound healing and scarring available. [6]

Cutaneous malignant melanoma is very aggressive and infamously resistant to chemotherapy, radiotherapy, and immunotherapy strategies. Partial spontaneous regression is observed in some forms of human melanoma, but only rarely does complete regression occur. By contrast, swine spontaneously develop melanoma analogous to that observed in humans that very frequently regresses completely. The Sinclair swine cutaneous melanoma (SSCM) model and the melanoma-bearing Libechev minipig (MeLiM) model have been developed and characterized in the hope of discovering genes that can be identified as susceptibility loci for heritable melanoma or associated with the cellular and molecular mechanisms involved in melanoma regression. [3,7,8] Genetic variability in four genes are already known to account for 50% of human familial malignant melanoma (FMM) cases. Further work on this issue is being performed using computer-assisted mathematical modeling using the SSCM model in the hope of identifying these and other candidate loci involved in the initiation, progression, and aggressiveness of FMM in swine. In this case the SSCM model provides advantages over similar familial studies in humans because of the short gestation times and high progeny numbers achievable with swine. [7] In the MeLiM swine model, tumors naturally regress with a frequency of 96% and are observed to become more flat, dry, depigmented, and infiltrated with leukocytes as they regress. Analysis of the swine transcriptome during tumor regression has identified a pool of approximately 1,400 genes that are differentially expressed during this process. Though obviously further review of each of these genes will be required, some interesting general trends are apparent. For example, genes involved in cell cycle and DNA replication and repair are down-regulated early in regression suggesting retardation of melanoma cell proliferation. Additionally, genes associated with monocytes/macrophages are up-regulated at intermediate time points during regression suggesting that tumors do not evade immune attack in swine. [8]

Diabetes

While mainstream models of diabetes research remain rodents, there has been an increasing need for translational research in large animal models of the disease. Swine have many similarities to humans that make them a good choice for modeling diabetes including an omnivorous diet, gastrointestinal tract anatomy and physiology, general metabolic status, and pancreatic size, shape, and position (particular in minipigs). Further, the fact that swine are closer to humans phylogenetically, as evidenced by swine and human insulin polypeptides differing by only one amino acid, is important when interpreting data or translating results to clinical applications. Another advantage of the swine model is their similar pharmacokinetics with respect to subcutaneously administered drugs. [2,9]

Swine models of type 1 diabetes (T1D), type 2 diabetes (T2D), and maturity-onset diabetes of the young type 3 (MODY3) have been developed. The swine T1D model is created by administering streptozotocin (STZ) to intact animals. STZ is preferentially lethal to the insulin-secreting β -cells in pancreatic islets of Langerhans and STZ-treated swine display a human-like diabetic phenotype as evidenced by their significantly increased blood glucose levels after both intravenous and oral glucose delivery. This model has been used for a variety of investigations into T1D pathology including recent proteomics studies that implicate several proteins as potential target candidates for further analysis. [9] Models of T2D include a new transgenic swine line in which pancreatic β -cells express a dominant negative mutant human *gipr* transgene. Discovery studies in mice have implicated glucose-dependent insulinotropic polypeptide (GIP) and its receptor (GIPR) in T2D pathogenesis. Normally, lipid- or glucose-induced GIP secretion from enteroendocrine cells promotes glucose-mediated insulin release. However, in T2D patients, GIP and/or GIPR are functionally impaired resulting in poor insulin response to oral glucose. Transgenic GIPR^{dn} swine display deficits in oral, but not intravenous, glucose tolerance resulting from poor insulin secretion at 11 weeks of age. By 5 months, these animals additionally displayed a marked reduction in total β -cell volume and by 11 months, intravenous glucose tolerance is also significantly impaired. These results confirm that GIP/GIPR are critically involved in the maintenance of β -cell health *in vivo* and thus further promote their status as potential T2D therapeutic targets. [2] A transgenic swine model of MODY3 has been developed in which a dominant negative mutation in the human hepatocyte nuclear factor 1 α (HNF1A) transgene is expressed. Like GIPR, HNF1A was first identified as a candidate target in mouse. While most transgenic HNF1A^{dn} piglets died shortly after birth, those that survived displayed elevated non-fasting blood glucose levels and transgenic protein expression in the pancreas and kidney. Upon closer examination, malformation of pancreatic islet structures and glomerular hypertrophy and sclerosis of the kidneys is evident. [2]

Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease resulting from a variety of mutations in the *cftr* gene, which codes for the cystic fibrosis transmembrane conductance regulator anion channel. The disease dramatically affects multiple organs, but the main cause morbidity and mortality in humans is failure of the lungs as a result of chronic inflammation and secondary infection. Despite intensive study, available therapies for CF are inadequate and the disease still has no cure. One of the main obstacles in CF research has been the lack of good animal models as mice expressing defective *cftr* genes fail to develop lung disease. In search of a better model in which to study CF pathogenesis and potential therapeutics, researchers have recently been focusing on swine. [2,10]

Swine have a lifespan amenable to the study of a disease like CF where lung deterioration progresses over a relatively long period of time and potential novel therapeutics would need to be tested over time as well. In contrast to mice, swine lungs are anatomically, physiologically, histologically, and biochemically very similar to human lungs. Further, swine have already been established as reliable animal models for a variety of lung-related studies including lung development, injury (hypoxia-, toxin-, or reperfusion-induced), growth after lobectomy, transplantation, airway hyper-responsiveness, asthma, surfactant biology, and many other aspects of lung pathophysiology. [10]

Transgenic swine bearing mutations in the *cftr* gene have recently been innovated and characterized as developing lung disease that very closely approximates clinical observations. Wild-type human and swine CFTR proteins display 93% identical amino acid sequences that are translated in the endoplasmic reticulum (ER), glycosylated in the Golgi, and transported to the apical membrane of airway epithelia where they function as anion channels controlled by phosphorylation. While human mutant CFTR protein is nearly completely retained within the ER and degraded, a very small portion of swine mutant CFTR escapes and can be detected on the apical membrane. Despite this low level CFTR presence, swine expressing mutant CFTR show aberrant airway epithelial electrolyte transport similar to humans. [10]

The lungs of patients suffering from CF easily become infected by a wide variety of pathogens and though humans and swine are not susceptible to precisely the same subset of viral or bacterial species, the resulting immune response and chronic inflammatory state that ensues is very comparable. Swine possess airway host defense mechanisms that are notably similar to humans including resident phagocytes expressing the full complement of pattern recognition receptors, soluble bioactive peptides and proteins including defensins, collectins, and others, and a wide variety of pro-inflammatory cytokines, chemokines, and their respective receptors. How CFTR dysfunction adversely affects host defense making sufferers more susceptible to infection is not known, but increasing ionic strength of human and swine airway surface liquid is known to reduce its antimicrobial capability. [10]

Traumatic Brain Injury, Stroke, and Neurodegenerative Disease

While swine have historically not been used as a model for the human central nervous system due to their enormous skull bones and vertebrae and very narrow intervertebral spacing, their appearance in the neuroscience literature has been rapidly increasing. Swine have relatively large brains that are anatomically more similar to humans than rodents in that they are gyrencephalic, contain more white matter, and have similar patterns of cerebral blood flow. [1]

Because swine also display a very human-like progression of early brain development, neonatal swine have become a particularly interesting new model of pediatric traumatic brain injury (TBI) and stroke. TBI is characterized by primary distortion of the parenchyma and secondary excitotoxicity, cell death, axonal injury, cerebral swelling, and inflammation. Swine and human responses to TBI are very similar and have been well characterized in terms of arterial and intracranial pressures, cerebral blood flow, histopathology, and a variety of other measures. Further, simple functional testing using a veterinary coma scale and complex neurobehavioral testing to assess functional outcome of TBI-treated swine are documented. This enables researchers to determine whether novel neuroprotective therapies offer significant improvement in neurological outcomes. [11] Ischemic stroke in pediatric patients is mechanistically different than observed in adults and thus suggests treatment regimens be age-specific. However, there is still relatively little known about stroke in children as options for animal models have been limiting. A new neonatal swine model reliably produces clinically-relevant ischemic stroke in both grey and white matter that upon histopathological analysis shows evidence of platelet activation,

thrombus formation, apoptosis, and localized accumulation of inflammatory leukocytes. The model also, as a result of the large size of piglets relative to rat pups for example, facilitates collection of other important physiological measurements including blood pressure and oxygen saturation. [12]

Large animal models are particularly important for the study of human neurodegenerative diseases as rodent models cannot approximate the size and gross anatomy of human brains much less their neuroanatomical connectivity and cognitive capacity. Monkey and swine are the most human-like neurological models described to date and are critical for bridging the gap in neurodegenerative disease research between basic discovery science performed in rodents and clinical trials for human therapeutics. Swine models of Parkinson's disease (PD) and Huntington's disease (HD) have been extensively studied and recently been used for the production of xenografts that are already in clinical trials. [13] Advances in swine genomics have resulted in the development of transgenic swine models of several other neurodegenerative diseases including HD, Alzheimer's disease (AD), and retinitis pigmentosa (RP). The transgenic swine HD model expresses mutant swine huntington protein (HTT) while the AD model expresses mutant human amyloid precursor protein (APP). Neither of these models have yet been fully characterized as functional deficits will only appear as the animals age. The transgenic swine RP model expresses mutant swine rhodopsin protein (RHO) and as a result is afflicted, like humans, with early and near complete degeneration of rod photoreceptors followed by a more protracted deterioration of cone photoreceptors. [2]

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