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Review Article

A REVIEW ON IN-VITRO AND IN-VIVO MODELS OF VITILIGO

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ABSTRACT

Vitiligo is an acquired, progressive depigmentary disorder that is characterized by the loss of functional melanocytes resulting in clear skin depigmented lesions. The pathogenesis of vitiligo is multifactorial and complex: despite the development of clinical and molecular research, the occurrence of oxidative stress, autoimmune deficiency, genetic predisposition, and an intrinsic vulnerability of melanocytes remain unaddressed. Powerful experimental models are thus needed in mechanistic exploration and therapeutic development. The review is a critical expression of the models presently used in vitiligo studies both in vitro and in vivo. various invitro models that are discussed include cytokine treated melanocyte models, oxidative stress and ferroptosis, keratinocyte-melanocyte interactions, 3D skin equivalents, ipsc-derived melanocyte. in vivo models include various mice models such as C57BL/6 mouse model, Human skin xenocraft mice, smyth line chicken model, UV-induced vitiligo rat model.

Keywords: Vitiligo, Depigmentation, In vitro models, In vivo models, Melanocyte.

INTRODUCTION

Vitiligo models have been rapidly evolving towards mechanism-based systems that epitomize immune, metabolic and microenvironmental associated changes which causes melanocyte loss. Current trends revolve around oxidative/ER stress, innate sensors, and type 1 IFN circuits linkage towards the destruction of CD8+ T cell and failed immune regulation. (Zhao *et al*., 2022; Seneschal *et al*., 2020). A translational framework linking human immunopathology to optimized murine models of melanocyte loss and relapse has been established by Harris and colleagues and implicates IFN g/JAK-STAT chemokine loops, CXCR3 ligands, and CCR5+ Tregs as central regulators of melanocyte loss and relapse (Long *et al*., 2025).

They highlight the interaction of keratinocytes, fibroblasts, innate lymphoid cells, and tissue resident memory T cells (T), which redefine models to incorporate non melanocytic and metabolic components. Recent Chinese groups have produced endogenous CD8+ and fibroblast driven models which predict in toto stromal IFN g responsiveness and bilateral symmetry (Cario-André *et al*., 2018).

Recent Major Advances in In Vivo Models

New reviews categorize mouse models into spontaneous, induced and transgenic models, although more significantly by mechanism of melanocyte loss and immune implication to inform model selection in the initiation vs. chronicity vs. therapy studies. Patchy epidermal depigmentation was first reproducibly induced in k14 SCF mice by the Riding/Harris protocol, which allowed lesion scoring to be quantified and drug testing to be done (Giri *et al*., 2024). SCF hairless hk14 mice now remove fur interference and closely recap human melanocyte localization; induction with PMEL CD8+ cells or PMEL TCR crosses result in disease that can be image quantified and has been tested to be UV, corticosteroids as well as JAK inhibitive. A model of endogenous vitiligo in C57BL/6 strains using transient exposure of B16F10 and depletion of CD4+ Tregs induces vitiligo in all strains of C57BL/6 beginning with day 19 and allowing subsequent study of early CD8+ infiltration and melanocyte loss (Riding *et al*., 2018). An IFN g responsive

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fibroblast-mediated melanoma/Treg model shows that IFN g responsive dermal fibroblasts coordinate CD8+ recruitment and symmetry; IFN g blockade suppresses vitiligo which provides a platform to fibroblast-based bispecific antibodies. In a broader context, reviews on animal models emphasize that no specific system is able to encompass all pathogenesis, but complementary models are able to do this (Xie *et al*., 2025).

Table 1. *In vivo* Experimental Models Used in Vitiligo Research.

Model Type	Induction Period	Treatment Period	Mechanism / Key Features	References
C57BL/6 Mouse – B16F10 Cell Lysate Sensitization	14–21 days	4–6 weeks	Induces $CD8^+$ T-cell–driven melanocyte destruction; models autoimmune response comparable to human vitiligo	Bogacka *et al*., 2022
Human Skin Xenograft on Immunodeficient Mice ($RAG2^{-/-}\gamma c^{-/-}$)	4 weeks engraftment	6–8 weeks	Allows in vivo testing of human melanocyte-targeting T-cells; reproduces autoimmune depigmentation via human immune reconstitution	Bast G. *et al*,2005
Smyth Line Chicken Model	6–8 weeks post-hatch	Chronic (lifelong)	Spontaneous autoimmune vitiligo model with strong genetic penetrance; exhibits parallel T-cell and cytokine dysregulation as in human disease	Shi F,*et al*,2012
UV-Induced Vitiligo Model (Rat)	10–14 days UV exposure	21 days	Depigmentation through oxidative stress and melanocyte apoptosis; validated for testing photoprotective and antioxidant interventions	Shi F,*et al*,2012

Recent Significant Progress in In Vitro /Ex Vivo Models

Simple monolayers have been replaced by tunable and scalable, in vitro 3D and iPSC-based work. Primary culture of classical melanocytes, keratinocytes and fibroblasts (usually patient derived) interrogates oxidative stress susceptibility, mitochondrial dysfunction, insulin/IGF 1 resistance, and fibroblast mediated melanocyte inhibition, explaining a metabolic-immune bridge(Chang and Ko,2023). A melanocyte death and dendrite retraction dose response in an iPSC derived melanocyte-keratinocyte co culture has been induced by exposure to graded IFN g and has been used to test ROCK inhibition, which reverses adhesion molecule levels and melanocyte survival placing ROCK inhibitors as a rational adjunct and the system as a scalable drug screening platform. Reviews In vitro vitiligo studies describe 3D organotypic skin cultures and co cultures of immune cells reconstituting networks of keratinocyte-melanocyte-fibroblast in which gene modification modalities can be used to bridge to transgenic animal studies. Lesional and non-lesional single cell RNA sequencing has emerged as a new de facto ex vivo model and indicates the involvement of complex subclinical immune activation in epithelial and immune cells and that CCR5 dependent positioning (and not recruitment) is essential to maintain disease control by Tregs, which has now been validated in mouse models (Gellatly *et al*.2021).

Table 2. In vitro Experimental Models Used in Vitiligo Research.

Model	Set-up	Endpoints	References
Cytokine-treated melanocytes	Normal human melanocytes are grown in culture and exposed to inflammatory cytokines such as TNF-α, alone or together with IL-6 or IL-10, for 24–48 hours	Loss of melanocyte survival, increased apoptosis, activation of inflammatory receptors (TNFR1/2), and increased expression of adhesion and immune signaling molecules (ICAM-1, IL-6)	Singh *et al*., 2014
Oxidative-stress model	Treat cultured melanocytes with H_2O_2 (200-500 μM) or ferroptosis inducers (erastin, RSL3).	ROS generation, lipid-peroxidation, iron accumulation, loss of viability; rescue by GPX4-up-regulating agents (e.g., baicalein).	Liu *et al*., 2025

Keratinocyte-melanocyte co-culture	Melanocytes are grown together with keratinocytes in direct contact or separated by a membrane; inflammatory or oxidative stimuli are added.	Release of chemokines by keratinocytes, impaired melanocyte growth and pigment production, increased melanocyte apoptosis	Lee AY, 2012
3-D skin equivalents (reconstructed epidermis)	A skin construct that is multilayered is developed having fibroblasts, keratinocytes, and melanocytes and subjected to depigmenting reagents or immune stimuli	visible melanin loss, skin destruction, and immune-mediated destruction of melanocytes.	Lyu & Sun, 2022
iPSC-derived melanocytes	Induced pluripotent stem cells of patients are differentiated into melanocytes and subjected to immune or oxidative stress. Patient-specific melanocytes are induced using human induced pluripotent stem cells and evaluated in immune or oxidative stress conditions	Altered pigmentation genes expression, enhanced sensitivity to immune killing, and protection by targeted therapy drugs, including JAK inhibitors	Li *et al*.2025
Ferroptosis-focused assay		Validation of ferroptosis as a melanocyte-destruction mechanism; testing of ferroptosis inhibitors.	Lee AY, 2012

Current Debates and Gaps

The main controversies revolve around the extent to which each of the models is faithful to the human heterogeneity. The CD8+ models have been shown to recapitulate nonsegmental, inflammatory vitiligo at the expense of segmental vitiligo, long standing stable disease, and psychosocial or metabolic precipitants. Models of depigmentation that occur under the influence of chemical and drugs model environmental insults, but might engage different injury pathways to those of idiopathic autoimmunity. Big animal models are also not common and therefore the investigation of human like skin architecture and chronicity is restricted. At the in vitro scale, the majority of 3D systems are yet to have autologous T cells, T_RM niches, neural and vascular units, and hair follicle melanocytes reservoirs, limiting relapse and repigmentation source modeling. They cannot easily be compared and standardized across models of specific questions (initiation vs. maintenance vs. therapy response), which makes cross study interpretation difficult.

Future Directions

The future work is moving towards integrated and multi scale modeling. The reviews of mouse models indicate the necessity of fine-tuned models that further reflect human heterogeneity, long-term relapse, and complex therapeutic responses, such as combination therapy that includes oxidative stress, innate sensors, IFN g/JAK-STAT, chemokine axes, TRM and metabolic defects. Humanized immune mice, hairless models with longitudinal images and fibroblast or keratinocyte models of intervention will prove important in testing cytokine, checkpoint and bispecific antibody strategies (Ezzedine *et al*.2025). In vitro, there is the priority of scalable patient derived 3D or organoid-like skin recapitulating autologous immune and stromal cells, with single cell and spatial omics guidance. These systems coupled with high content screening must enhance the translation of JAK inhibitors, ROCK and WNT modulators, antioxidant and metabolic therapies and targeted biologics into long-lasting, personalized repigmentation strategies (Komatsu *et al*.2025).

CONCLUSION

In vivo and in vitro models of vitiligo have been replaced with non-descriptive mechanistically defined stages of immune, stromal and metabolic regulation capable of dissecting immune, stromal and metabolic checkpoints. Endogenous autoimmunity mediated by CD8+ T cells, hairless and fibroblast focused mouse models, sophisticated human cell and iPSC-based systems, have directly informed JAK, cytokine, checkpoint, ROCK and antioxidant-based therapeutics. The primary issue in the field is not that there are no models but that the heterogeneity and chronicity of the diseases has not been fully covered, and that the integrations across platforms have not been fully made. It will be necessary that systematic, question guided use and refinement of models-

grounded to human multi omics- be used to improve predictive capacity and that restitution of predictive capacity will result in predictability of a stable, relapse free repigmentation in vitiligo.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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