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*Review*

# Advances in the Study of Zebrafish Hemodynamic Models

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**Abstract**

Zebrafish (*Danio rerio*) have emerged as a leading vertebrate model for cardiovascular research, offering unique advantages such as optical transparency, genetic tractability, and high fecundity. This review provides a comprehensive synthesis of the roles of hemodynamic forces, including wall shear stress (WSS), pressure gradients, and the oscillatory shear index (OSI), in regulating cardiovascular development, homeostasis, and disease pathogenesis. We describe the integration of advanced *in vivo* imaging modalities, such as light-sheet microscopy, high-frequency ultrasound, and high-speed particle-tracking velocimetry, with computational fluid dynamics (CFD) and fluid-structure interaction (FSI) models. These synergistic approaches enable precise quantification of biomechanical parameters and elucidation of the mechanobiological signaling pathways that transduce hemodynamic stimuli into cellular responses, particularly the Notch and Wnt cascades. The utility of zebrafish in modeling congenital heart defects, cardiomyopathies, vascular malformations, and vascular metabolic diseases is also highlighted, including diabetic retinopathy, thrombosis, and hyperlipidemia, alongside their critical role in high-throughput drug screening and mechanistic studies of cardioprotective agents. Finally, we address current limitations related to anatomical differences and scaling effects, and propose future directions in multi-omics integration, patient-specific modeling, and artificial intelligence (AI)-driven analytics to enhance translational relevance.

**Keywords:** zebrafish; hemodynamics; models, cardiovascular; mechanotransduction, cellular; drug evaluation, preclinical; heart defects, congenital

## 1. Introduction

### *1.1 Advantages of Zebrafish in Hemodynamic Research*

The zebrafish (*Danio rerio*) is a powerful vertebrate model for cardiovascular research, facilitating the study of hemodynamic forces in development and disease [1,2,3]. A key biological feature is its optical transparency, enabling non-invasive, real-time visualization of cardiac and vascular dynamics via brightfield or fluorescence microscopy [1,4,5,6]. Combined with rapid external development and high fecundity, this allows for large-scale, high-throughput screening impractical in other vertebrates [1,2,3]. Its survival without an active circulatory system in early stages further aids genetic and pharmacological manipulation [1,4].

Genetically, the zebrafish shares a high degree of genetic homology with humans, and its sequenced genome permits the induction of clinically relevant defects via CRISPR-Cas9, elucidating conserved molecular pathways [1,3,7,8,9]. Technically, its small size and external development facilitate chemical exposure and microinjection [2,3]. Critically, the integration of advanced imaging with computational fluid dynamics (CFD) modeling provides detailed insights into blood flow patterns and hemodynamic stress; these imaging modalities include light-sheet microscopy and high-speed cameras adapted for particle tracking velocimetry [1,4]. These combined attributes make zebrafish indispensable for hemodynamic research.

### *1.2 Scope and Significance of Hemodynamic Research in Zebrafish*

Hemodynamics, the study of blood flow and associated forces, is central to cardiovascular development, disease, and therapy. In zebrafish, hemodynamic forces actively sculpt organogenesis and drive tissue remodeling rather than serving as passive consequences of the heartbeat [10]. Shear stress critically influences vascular development and cardiac valve formation, with perturbations leading to malformations [11,12]. The optical transparency of the zebrafish allows real-time visualization of these mechanobiological interactions [1,12].

Regarding pathogenesis, altered hemodynamics drive cardiovascular disorders. For instance, *heg1* knockout mutants recapitulate human heart failure and thrombosis phenotypes [13], while medullary thyroid carcinoma models reveal tumor-induced vascular permeability changes [14]. These models elucidate mechanisms from congenital defects to vascular pathologies.

The zebrafish also serves as a high-throughput platform for therapeutic screening. Evaluations of Traditional Chinese Medicine (TCM) compounds have demonstrated

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improved microvascular hemodynamics [15], while agents like ELABELA show cardioprotective effects against hypertrophy via the renin-angiotensin system [16]. Furthermore, heart rate (HR) and heart rate variability (HRV) provide crucial endpoints for preclinical cardiotoxicity assessment [17]. By bridging conserved molecular pathways with clinical phenotypes, zebrafish research accelerates the translation of mechanobiological insights into diagnostic and therapeutic strategies.

### *1.3 Framework of This Review*

To provide a coherent and systematic overview, this review is organized around a central logic chain that moves from technology to quantification, mechanism, and finally application. Section 2 introduces the technological foundations, namely imaging modalities and computational methods, that make hemodynamic studies possible. Section 3 discusses how the resulting biomechanical parameters such as wall shear stress (WSS) and pressure regulate cardiovascular development through specific mechanobiological pathways. Section 4 reviews the application of these principles in disease modeling. Section 5 focuses on the quantitative assessment of cardiac function and flow dynamics. Section 6 evaluates the platform for drug screening, and Section 7 critically examines current limitations and future opportunities. This framework aims to provide both a comprehensive reference and a critical perspective on the field.

## 2. Technological Foundations for Hemodynamic Model Construction

### *2.1 Imaging Techniques for Hemodynamic Visualization*

Zebrafish hemodynamic research utilizes a complementary suite of *in vivo* and *ex vivo* imaging modalities to quantify cardiovascular dynamics. For adult zebrafish, where optical transparency is lost, high-frequency ultrasound (HFUS) echocardiography operating at up to 50 MHz is essential for assessing cardiac function and hemodynamics [18,19]. HFUS enables non-invasive measurement of key parameters such as heart rate, stroke volume, and ejection fraction and is pivotal for modeling age-related conditions and drug screening [18]. Recent advancements include ultrafast HFUS for myocardial strain imaging during heart regeneration [20] and high-frequency micro-Doppler imaging (HFμDI) for visualizing peripheral vessels with a minimum detectable diameter of 36 μm and a maximum imaging depth of 4 mm [21].

In transparent embryonic and larval stages, optical imaging dominates. Confocal microscopy facilitates 3D rendering of complex vascular networks in transgenic lines [22], while light-sheet microscopy (LSM) provides real-time, high-resolution 3D imaging of the beating heart with minimal phototoxicity [23,24]. LSM is critical for quantifying mechanobiological forces like WSS [25], and new cost-effective scan units now allow real-time stereoscopic imaging [26].

Table 1 summarizes the key imaging modalities, their applicable stages, measurable parameters, and advantages and limitations, thereby providing a quick-reference comparison for readers.

#### Direct Measurement of Blood Flow Velocity

While the above techniques excel at resolving structural morphology, directly measuring the velocity field of blood flow is essential for quantifying hemodynamic forces. Particle image velocimetry (PIV) and particle tracking velocimetry (PTV) have become increasingly powerful in the zebrafish model. These methods exploit the natural contrast of red blood cells (RBCs) or fluorescent labeling techniques; for instance, the Tg(gata1:dsRed) transgenic line brightly marks erythrocytes, and high-speed camera imaging can then capture RBC movement at high spatiotemporal resolution. Maung Ye et al. (2022) [27] utilized PTV of RBCs in zebrafish from 2 to 6 days post-fertilization (dpf) to map the developmental trends of flow speed, estimated WSS, viscosity, and hematocrit across the trunk vasculature, developing a semi-automated high-throughput system capable of imaging up to 50 zebrafish simultaneously. Similarly, Roustaei et al. (2022) [28] employed PTV to reconstruct a 4D microcirculatory network model, analyzing WSS dynamics and flow circulation during tail regeneration stages in 5 dpf larvae. These particle-based methods provide critical experimental inputs for computational models and offer a direct window into the physical forces experienced by cells.

*Ex vivo* techniques provide ultra-high-resolution structural context. Synchrotron X-ray micro-tomography resolves whole fixed zebrafish at cell-level resolution, with isotropic voxels of ~1 μm, providing histology-like three-dimensional context without physical sectioning [29], while chemical clearing methods like CUBIC enable detailed 3D mapping of cardiac vasculature and lymphatic vessels without sectioning [30,31]. The integration of these diverse modalities extends from HFUS in adults to advanced optics in embryos, high-speed flow velocimetry, and high-resolution *ex vivo* methods and constitutes a comprehensive toolkit for hemodynamic analysis across developmental stages.

### *2.2 Numerical Methods for Computational Hemodynamic Modeling*

Beyond visualization, computational modeling is indispensable for quantifying parameters difficult to measure experimentally. The choice of numerical method depends on the specific biological question and the required balance between accuracy and computational cost. Table 2 provides an overview of the principal numerical methods employed in zebrafish hemodynamic research.

Grid-based methods are widely used for solving the Navier-Stokes equations governing fluid motion. The Finite Volume Method (FVM) is popular for its conservation

**Table 1. Comparison of imaging modalities for zebrafish hemodynamics**.

| Modality | Applicable stage | Key measurable parameters | Resolution | Key advantages | Key limitations |
|---|---|---|---|---|---|
| Confocal microscopy | Embryo, larva | 3D vascular architecture, cell dynamics | ~200–500 nm (lateral) | Excellent spatial resolution; transgenic line-compatible | Limited penetration depth; moderate phototoxicity |
| Light-sheet microscopy (LSM) | Embryo, larva | 4D cardiac motion, WSS distribution, trabeculation | ~300–500 nm (lateral) | High speed, low phototoxicity; ideal for live time-lapse | Sample mounting complexity; post-processing demands |
| High-speed PTV/PIV | Embryo, larva (2–6 dpf) | Flow velocity, WSS, hematocrit, viscosity | Depends on camera frame rate | Direct flow measurement; quantifiable forces | Requires adequate RBC contrast and a high frame rate; fluorescent labelling (e.g., Tg(gata1:dsRed)) is optional, for fluorescence-based setups |
| High-frequency ultrasound (HFUS) | Adult | Heart rate, stroke volume, ejection fraction, vessel diameter | ~30 μm (axial) | Non-invasive; clinically translatable parameters | Lower spatial resolution; limited in embryos |
| Synchrotron X-ray tomography | *Ex vivo* (larva and juvenile; 4–33 dpf) | Cell-level 3D anatomy (virtual histology) | Nominal isotropic voxel edge length: 0.743 μm (larva, 4 dpf); 1.43 μm (juvenile, 33 dpf) | Ultra-high resolution; no sectioning required | Limited accessibility; *ex vivo* only; requires contrast staining |

PTV, particle tracking velocimetry; PIV, particle image velocimetry; WSS, wall shear stress; RBC, red blood cell; LSM, light-sheet microscopy; HFUS, high-frequency ultrasound.

**Table 2. Comparison of computational modeling approaches for hemodynamics**.

| Method category | Specific technique | Best-suited biological application | Key advantages | Key limitations |
|---|---|---|---|---|
| Grid-based CFD | Finite Volume (FVM), Finite Element (FEM), Finite Difference (FDM) | Macroscopic flow in heart/vessels; WSS and pressure field prediction | Robust conservation properties (FVM); handles complex geometries (FEM) | Difficult with large deformations; re-meshing needed for FSI |
| Lattice Boltzmann (LBM) | LBM + Immersed Boundary Method (IBM) | Cellular-scale blood flow; RBC dynamics in microvessels | Efficient for multiphase/ multicomponent flows; scalable | Limited to low-Mach-number flows; boundary condition complexity |
| FSI – Monolithic | Arbitrary Lagrangian-Eulerian (ALE) | Heart valve mechanics; moderate-deformation wall motion | High accuracy at fluid-solid interface | Computationally expensive; fails under extreme deformation |
| FSI – Partitioned | Immersed Boundary Method (IBM) | Large-deformation FSI; numerous deformable cells (RBCs) | No dynamic re-meshing; robust for complex geometries | Lower interface accuracy; requires dense Lagrangian marker distribution |
| Mesh-free Particle | Dissipative Particle Dynamics (DPD), Smoothed Particle Hydrodynamics (SPH) | Cellular-scale blood suspension; plasma-RBC ensemble simulation | Naturally handles multiphase flow and large deformations; no mesh generation | High computational cost; parameter calibration challenging |

CFD, computational fluid dynamics; FSI, fluid-structure interaction; FVM, finite volume method; FEM, finite element method; FDM, finite difference method; LBM, lattice Boltzmann method; IBM, immersed boundary method; ALE, arbitrary Lagrangian-Eulerian; DPD, dissipative particle dynamics; SPH, smoothed particle hydrodynamics; WSS, wall shear stress; RBCs, red blood cells.

properties, while the Finite Element Method (FEM) excels at handling complex geometries and often serves as the basis for FSI simulations [32]. The Finite Difference Method (FDM) offers a simpler but less flexible approach for structured grids.

For modeling blood at the cellular level, the Lattice Boltzmann Method (LBM) solves the Boltzmann transport equation, which under certain conditions is equivalent to the Navier-Stokes equations. LBM is highly efficient for simulating multiphase and multicomponent flows, making it suitable for resolving RBC dynamics in microvessels. It is often coupled with the Immersed Boundary Method (IBM) to handle deformable cells. Seminal works by Zhang et al. (2008) [33] and Dupin et al. (2007) [34] established LBM-IBM frameworks for simulating RBC behavior in shear flows and dense suspensions of deformable particles, respectively. These studies laid the methodological foundation for modern cell-and-plasma transport models [35].

When one must address deformable structures like heart valves or vessel walls, FSI modeling is required. In an Arbitrary Lagrangian–Eulerian (ALE) formulation the computational mesh moves with the domain boundary. Lee et al. [36] used image-derived, prescribed wall motion in an ALE-based CFD model of the embryonic zebrafish heart. This conforming-mesh strategy offers high accuracy for moderate deformations and should be distinguished from two-way fluid–structure interaction, in which structural deformation is solved together with its interaction with the fluid. For problems involving large, complex deformations or numerous moving cells, the immersed boundary method (IBM) provides an alternative to body-fitted moving meshes. In the formulation described by Peskin, immersed structures are represented by Lagrangian markers coupled to a fixed Eulerian fluid grid, so that no mesh conforming to the moving structure is required [37]. IBM has since been applied to three-dimensional fluid–structure interaction simulations of the adult zebrafish aortic valve [38]. Alternatively, particle-based, mesh-free methods like Dissipative Particle Dynamics (DPD) and Smoothed Particle Hydrodynamics (SPH) offer compelling approaches for modeling blood at the cellular scale, simulating plasma and RBCs as an ensemble of interacting particles. The DPD framework, extensively developed by Karniadakis, Fedosov and colleagues [39,40], has been successfully applied to predict human blood bulk viscosity and model RBC mechanics down to the spectrin level. The selection and application of these diverse numerical tools are driven by the specific hemodynamic parameters one aims to quantify.

## 3. Hemodynamic Mechanisms in Cardiovascular Development

### *3.1 Mechano-Biological Interactions During Cardiovascular Morphogenesis*

Cardiovascular morphogenesis is driven by a dynamic interplay of genetic programs and mechanical forces, with hemodynamics serving as a critical regulator from early development, specifically through WSS and pressure [11,41,42]. In zebrafish, hemodynamic forces actively guide processes including cardiac tube looping, chamber formation, and valve development rather than being passive consequences of flow [41,43]. WSS serves as a crucial mechanical cue for endothelial cells (ECs); for instance, spatiotemporal WSS variations regulate trabeculation, where pulsatile shear correlates with Notch activity along ridges while oscillatory shear develops in grooves by 4 dpf [41,44,45]. Genetic reductions in WSS attenuate oscillatory shear and disrupt ventricular maturation, underscoring hemodynamics' central role [45].

Microvascular remodeling is similarly governed by hemodynamics. RBC dynamics establish regional WSS gradients essential for vascular stability; computational and live-imaging studies link vessel regression to RBC hypoperfusion and plasma skimming, where RBC distribution modulates effective viscosity and endothelial WSS [46]. Beyond flow-derived shear, cardiovascular morphology is also reshaped by lesions acting on the myocardium and endothelium themselves: zebrafish TRα1 mutations cause atrial dilation and contractility defects linked to sarcomere disorganization [47], while ADTRP deficiency leads to vascular malformations and increased permeability through aberrant Wnt/β-catenin signaling [11,48].

These mechanical forces interface with cellular signaling. A well-characterized mechanosensitive cascade involves the Notch pathway, which is upregulated by vortical flows during early trabeculation and valvulogenesis [44]. In parallel, flow-induced Wnt/Ang2 signaling regulates vascular stability [48]. The endothelial metabolic state provides another layer of regulation: laminar flow directs pentose phosphate pathway activity to sustain extracellular matrix synthesis [49]. More recently, the mechanosensitive channel Piezo1 has emerged as a primary sensor of shear stress, controlling EC proliferation and alignment [50]. These diverse pathways collectively translate physical forces into the biochemical programs governing cardiovascular morphogenesis.

### *3.2 Distinction Between WSS- and Pressure-Induced Vascular Remodeling*

A critical nuance often overlooked is that vascular remodeling is driven by two distinct hemodynamic signals, namely WSS and blood pressure-induced circumferential stretch. These forces activate different mechanosensors and downstream pathways, leading to complementary yet distinct cellular responses. WSS, a frictional force exerted by flowing blood, is sensed at the apical surface of ECs by the glycocalyx [51] and by Piezo1 channels [52]. These sensors promote flow-aligned, stabilized phenotypes through KLF2- and eNOS-dependent signaling [53,54]. In contrast, blood pressure generates circumferential tension in the vessel wall, which is sensed by integrin-mediated focal ad-

hesions and the cerebral cavernous malformations (CCM) complex [55,56]. Dessalles et al. (2025) [55] demonstrated that pressure-induced increases in EC tension drive the circumferential reorientation of actin filaments to regulate endothelial tube diameter. In developing zebrafish, Chen et al. (2025) [56] showed that at 2 dpf, ECs in intersegmental vessels initially display circumferential actin bundles anchored by CCM1/Krit1, coinciding with the onset of blood pressure-driven vessel lumen expansion. As flow speeds increase from 2 to 4 dpf, this actin network transitions to a flow-parallel and isotropically meshed arrangement [56]. These findings reveal a developmental switch and a potential tug-of-war between pressure- and WSS-induced actomyosin mechanisms that collectively govern vessel formation and stabilization.

### *3.3 Hemodynamic Regulation of Vascular Maturation*

Vascular maturation is driven by hemodynamic forces that regulate EC metabolism, vascular mural cell (vMC) recruitment, and extracellular matrix composition. ECs sense WSS, which modulates their metabolic state; specifically, laminar flow maintains the oxidative pentose phosphate pathway (oxPPP) to produce ribose-5-phosphate, thereby driving elastin expression and subsequent vMC coverage [49]. Hemodynamics also guide vMC recruitment via a CXCR3/CXCL11 signaling network regulated by blood flow; inhibition of Cxcr3 in zebrafish disrupts pericyte association and causes vascular overgrowth, highlighting its role in stabilization [57]. Furthermore, flow directly influences pericyte proliferation through the mechanosensitive channel Piezo1 and downstream Notch signaling, which is crucial for cerebrovascular development [50]. Functionally, mural cells acquire vasoactivity early: pericyte-covered vessels constrict and dilate by 4 dpf, while vascular smooth muscle cells (vSMC)-covered vessels mature later at 6 dpf, with genetic ablation confirming active diameter regulation [58].

## 4. Hemodynamic Models in Cardiovascular Disease Research

### *4.1 Congenital Heart Disease and Cardiomyopathy Models*

Zebrafish models are instrumental in dissecting the etiologies of congenital heart disease (CHD) and cardiomyopathies. Their utility spans from mechanistic studies linking genetic defects to aberrant flow to serving as platforms for functional phenotyping (Table 3). For dilated cardiomyopathy (DCM), a transgenic line expressing antisense cardiac troponin C (cTnC) recapitulated human phenotypes including ventricular enlargement and reduced ejection fraction, thus serving as a model for studying how contractility defects lead to altered hemodynamics [59]. Recent work identified centrosome reduction defects as a cause of infantile DCM; knockdown of *RTTN* in zebrafish confirmed its conserved role in cardiac structure, demonstrating how cellular architectural defects drive hemodynamic failure [60].

Hemodynamic alterations also mediate environmental and structural defects. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces malformations like altered looping and ventricular standstill [61], while myosin heavy chain 6 mutations result in single-ventricle hearts, revealing that intracardiac flow regulates valve morphogenesis independently of contractility [62]. Mechanical forces are crucial for valve development; in the zebrafish atrioventricular canal, flow-dependent nuclear factor of activated T cells (NFAT) signaling in the endocardium is required for proper valve-leaflet delamination, and its loss produces thickened, hyperplastic valves [63]. Advanced tools like optical elastography further allow non-invasive quantification of cardiac pressure and contractility, detecting subtle functional deficits in CHD models [64]. These models collectively demonstrate how genetic and environmental insults converge on hemodynamics to produce disease phenotypes.

### *4.2 Vascular and Metabolic Disease Applications*

Zebrafish models effectively elucidate hemodynamic alterations in vascular and metabolic diseases. In diabetic retinopathy (DR) models, hyperglycemia induces hyaloid-retinal vessel dilation, which is mitigated by aldose reductase inhibitors like caffeoylquinic acids (CQAs) [65]. Hypoxia-driven angiogenesis is also modeled, with microglial extracellular vesicles and noscapine shown to modulate vessel sprouting and hemodynamic instability [66,67].

For thrombosis, the zebrafish offers a conserved hemostatic system; laser-induced models reveal that compounds like 6′-O-caffeoylarbutin (CA) inhibit clot formation [68,69]. Hyperlipidemia models demonstrate that lipid deposition preferentially occurs in low-WSS regions [70,71]. Therapeutic interventions such as 2-acetamidophenol (2-AAP), protocatechualdehyde (PCA)/hydroxysafflor yellow A (HSYA), and ezetimibe improve blood flow, reduce lipid accumulation, and enhance reverse cholesterol transport [72,73,74]. Finally, the zebrafish illuminates hypoxia-induced vascular remodeling, from cerebral vasoconstriction mitigated by nitric oxide generation [75] to VHL disease models showing HIF-driven neovascularization [76].

#### Vascular Malformation Models

The zebrafish has become invaluable for dissecting the hemodynamic underpinnings of vascular malformations. Hereditary hemorrhagic telangiectasia (HHT), characterized by arteriovenous malformations (AVMs), has been successfully modeled by Snodgrass et al. (2023) [77], who characterized vascular phenotypes in embryonic and adult endoglin mutant zebrafish. Their work revealed how disruption of BMP/TGF-β signaling leads to aberrant flow-driven shunt formation [77]. In the case of cerebral cavernous malformations (CCM), Rödel et al. (2019) [78] leveraged zebrafish to provide a mechanistic expla-

**Table 3. Summary of zebrafish cardiovascular disease models and their primary application focus.**

| Disease category | Specific model | Induction method | Core hemodynamic phenotype | Primary application focus |
|---|---|---|---|---|
| Dilated Cardiomyopathy (DCM) | cTnC antisense transgenic | Genetic (antisense cTnC) | Ventricular enlargement, reduced ejection fraction | Mechanistic: linking contractility defects to hemodynamic failure |
| TCDD-induced CHD | 2,3,7,8-TCDD exposure | Environmental toxin | Altered looping, ventricular standstill | Phenotyping: environmental insult leads to hemodynamic defect |
| Diabetic Retinopathy | Hyperglycemia-induced | Glucose immersion | Hyaloid-retinal vessel dilation | Screening: aldose reductase inhibitor testing |
| Thrombosis | Laser-induced endothelial injury | Physical (laser ablation) | Clot formation at injury site | Screening: antithrombotic compound evaluation |
| Hyperlipidemia | High-cholesterol diet/egg yolk feeding | Dietary | Lipid deposition in low-WSS regions | Mechanistic & Screening: linking WSS to atherosclerosis; drug testing |
| HHT/AVM | *endoglin* mutant | Genetic (mutant line) | Arteriovenous shunt formation, skin/retinal AVMs | Mechanistic: BMP/TGF-β signaling leads to flow-driven malformation |
| CCM | *ccm1* deficient + cardiac function rescue | Genetic + physiological restoration | Hyperplastic vascular expansion in low-flow regions | Mechanistic: blood flow as protective factor in lesion pathogenesis |

TCDD, tetrachlorodibenzo-p-dioxin; CHD, congenital heart disease; HHT, hereditary hemorrhagic telangiectasia; AVM, arteriovenous malformation; CCM, cerebral cavernous malformation; BMP, bone morphogenetic protein; TGF-β, transforming growth factor-beta.

nation for why human lesions predominantly form in low-perfusion venous capillaries. By restoring cardiac function and blood flow in a CCM1-deficient model, they demonstrated that blood flow is protective against the hyperplastic vascular expansion characteristic of this pathology [78]. These models enable high-resolution observation of how genetic defects initiate structural abnormalities that then create pathological hemodynamic microenvironments and perpetuate disease progression. This represents an ideal context for studying the causal relationship between mechanics and malformation.

## 5. Quantitative Measurement and Analysis of Hemodynamic Parameters

### *5.1 Heart Rate and Rhythm Assessment Techniques*

Accurate measurement of HR and HRV is critical for assessing zebrafish cardiac function and cardiotoxicity. Given the limitations of manual counting, automated high-throughput methods are essential. Given the limitations of manual counting, automated and semi-automated image analysis provides useful quantitative approaches, including semi-automated analysis of high-speed optical recordings and digital motion analysis of the peripheral vasculature [79,80]. Dedicated software such as HeartBeat enables label-free, high-throughput screening in microtiter plates, while Z-HRAM uses multiresolution optical flow to detect larval heartbeats from body deformation in low-resolution videos [6,81]. OpenCV-based approaches further allow for robust region-of-interest detection [1].

Complementing motion tracking, genetically encoded calcium biosensors such as GFP-aequorin permit *in vivo* assessment of cardiac calcium handling: in Tg(myl7:GA) larvae, terfenadine-induced cardiac dysfunction was characterised by bradycardia, 2:1 atrioventricular block and reduced ventricular calcium levels [82]. In adult zebrafish, ventricular electrical gradients underlying ECG patterns have been characterised under baseline conditions and oxidative stress [83]. An image- and CRISPR/Cas9-based study identified hcn4 as a contributor to heart rate, HRV, and susceptibility to sinoatrial pauses and arrests [84]; HRV analysis indicates that intrinsic cardiac properties govern early development, and reduced HRV has been linked to congenital defects such as cmlc2 mutations [6,8]. Together, these approaches provide complementary measurements of cardiac rate, rhythm, calcium handling and electrical function, and facilitate the quantification of AV delay and impulse velocity [4]. These assays are also indispensable for drug discovery, enabling rapid characterisation of chronotropic effects and cardiotoxicity in transgenic embryos [2,3,9,11].

### *5.2 Flow Dynamics and Biomechanical Parameter Quantification*

Quantifying hemodynamic forces to decode mechanobiological interactions in zebrafish cardiovascular development requires the precise measurement of wall shear stress (WSS), Reynolds number (Re), and oscillatory shear index (OSI). This quantification relies on the synergistic integration of experimental flow imaging and computational modeling, as detailed in Section 2.

The scientific value of these integrated approaches extends far beyond measuring parameters that are experimentally inconvenient to access. These models serve three distinct purposes. First, they are descriptive, mapping the spatial and temporal distribution of forces like WSS during development [25,85]. Second, they are mechanistic, testing hypotheses by perturbing parameters and observing the flow response; an example is elucidating how RBC partitioning and hematocrit variations modulate network WSS and pressure gradients during remodeling [35]. Third, they are predictive, forecasting the hemodynamic outcome of a pharmacological or genetic intervention. For instance, computational modeling has been used to quantify the bending stiffness of endothelial cilia *in vivo*, thereby predicting their mechanosensory properties [86]. The trend is moving towards specimen-specific, multi-scale models that integrate molecular-level rheological changes with organ-level flow. This provides a comprehensive platform for both fundamental discovery and translational screening.

## 6. Drug Screening and Therapeutic Evaluation Using Hemodynamic Models

### *6.1 Cardioprotective Drug Efficacy Assessment*

Zebrafish hemodynamic models serve as a cost-effective platform for preclinical drug screening, enabling rapid assessment of cardiovascular parameters like cardiac output and vascular tone. These models are pivotal for evaluating heart failure therapies including SGLT2 inhibitors. In a diabetic heart failure model, empagliflozin (EMPA) and sotagliflozin (SOTA) improved contractility and survival, though SOTA exhibited dose-dependent toxicity manifesting as pericardial edema at higher concentrations [87]. TCMs are also effectively evaluated; for instance, Dang-Gui-Si-Ni (DGSN) decoction enhanced cardiac output and blood flow velocity while downregulating coagulation factors in a thrombosis model [15]. Beyond targeted therapies, deep-learning-assisted phenotypic screening identified cyanidin chloride (CyCl) as a potent suppressor of doxorubicin-induced cardiotoxicity [88]. Additionally, the zebrafish facilitates hypolipidemic drug testing via egg yolk-induced hyperlipidemia models, validating drugs like simvastatin and natural products like Pu-erh tea [89]. These high-throughput platforms serve as screening engines, yielding quantitative hemodynamic readouts that accelerate the discovery and initial prioritization of cardioprotective candidates.

### *6.2 Mechanistic Studies of Drug Action*

Zebrafish hemodynamic models are pivotal for elucidating the mechanistic basis of therapeutic agents, moving beyond simple functional readouts to dissect intracellular targets. A key example is the cardioprotection by SGLT2 inhibitors EMPA and SOTA and cariporide, which was shown to involve the inhibition of sodium-hydrogen exchanger 1 (NHE1) to normalize intracellular $Na^+$ and $Ca^{2+}$ levels [87]. In the context of mitochondrial dysfunction, zebrafish models have identified visnagin and diphenylurea as binders of mitochondrial malate dehydrogenase (MDH2) [90], while Linggui Zhugan Decoction (LGZGD) was found to modulate the AMPK-FOXO3a pathway via BTG2 to preserve mitochondrial integrity [91]. Furthermore, an isosteviol derivative activates SIRT3 to upregulate SOD2 and OPA1 [92], and baicalein mitigates endoplasmic reticulum stress and apoptosis [93]. At the systemic level, the Renin-Angiotensin-Aldosterone System (RAAS) is effectively modeled; fimasartan improved fractional shortening and survival in arrhythmia-induced heart failure by reducing apoptosis [94]. Finally, the optical transparency of larvae facilitates the study of cerebral hemodynamics and neurovascular coupling, providing a platform for investigating neuroprotective strategies against stroke and neurodegeneration.

## 7. Challenges and Future Directions in Zebrafish Hemodynamic Research

### *7.1 Current Limitations of Zebrafish Models*

Despite the advantages of zebrafish, inherent limitations must be acknowledged when extrapolating findings to human clinical applications, particularly concerning parameter interpretation and translational fidelity.

Imaging adult zebrafish and performing physiological measurements is technically challenging [95]. Beyond these general constraints, the biological differences profoundly affect data interpretation. The two-chambered heart, while simplifying analysis, generates intracardiac flow patterns and pressure gradients fundamentally different from the four-chambered human heart. Thus, WSS patterns observed during valvulogenesis may not be directly mapped onto human physiology [95]. The zebrafish also operates in a low-Reynolds-number hemodynamic regime. Although this ensures laminar flow, it means that WSS magnitudes and biological scaling are not linearly translatable to the high-Re, pulsatile environment of the human aorta [70]. Genetic redundancy from teleost-specific genome duplication further complicates mechanistic studies because paralogs can mask phenotypes, leading to an underestimation of a gene's role in hemodynamic responses or disease [95,96]. While computational models help bridge this gap, the biological consequences of these distinct physical environments require careful, quantitative scrutiny rather than qualitative assumption.

Technical challenges also persist. High-throughput imaging, critical for drug screening, is often bottlenecked by the laborious and skill-intensive mounting and positioning of larvae for stable image acquisition. Current protocols remain semi-automated, limiting the transition to true big-data paradigms.

### *7.2 Emerging Opportunities and Innovations*

To overcome these limitations, the field is poised for transformation through the convergence of multiple cutting-edge technologies.

Multi-omics integration can resolve how complex hemodynamic environments shape molecular landscapes systems-wide. Consider an experiment where ECs are sorted from high-WSS versus low-WSS vascular regions, followed by paired RNA-seq and ATAC-seq. This would generate a mechano-epigenomic map and identify transcription factors like Klf2 that open chromatin to orchestrate a flow-protective gene expression program. Such data, integrated with high-resolution WSS maps, would enable the construction of gene regulatory networks that directly respond to flow dynamics, offering novel therapeutic targets for flow-sensitive diseases like atherosclerosis.

Patient-specific modeling is another frontier. The vision is a closed loop wherein a patient's specific genetic mutation, for instance in a contractile protein, is introduced into a zebrafish via CRISPR-Cas9. The resulting cardiac function and hemodynamics are then quantified, and the fish serves as an "avatar" for testing a panel of drugs tailored to that specific mutation [97].

Advanced AI and machine learning will be the unifying engine for these advancements [98]. AI-driven automation of sample handling, imaging, and image analysis could finally break the high-throughput bottleneck, enabling true big-data cardiovascular screening. Deep learning algorithms can process raw video data to instantly segment vessels, track RBC velocities, and calculate WSS across hundreds of embryos simultaneously. However, challenges remain in model generalization across diverse genetic lines and the interpretability of AI-driven phenotypes in biological terms. By evolving towards comprehensive, patient-centric, and data-rich platforms, zebrafish models can become even more predictive tools for identifying and personalizing cardiovascular therapies.

## 8. Conclusion

In summary, the zebrafish has solidified its position as an indispensable vertebrate model for hemodynamic research, uniquely bridging the gap between *in vitro* systems and mammalian physiology. This review has synthesized how its inherent advantages namely optical transparency, genetic tractability, and high fecundity converge with an expanding toolkit of advanced *in vivo* imaging and computational modeling methods. This toolkit encompasses light-sheet microscopy, high-frequency ultrasound, particle tracking velocimetry, and a spectrum of CFD and FSI approaches. These methods enable the precise quantification and mechanistic dissection of biomechanical forces like wall shear stress, pressure gradients, and oscillatory shear. We have detailed how these forces, through pathways such as Notch, Wnt, and Piezo1, actively govern cardiovascular development, vascular maturation, and the pathogenesis of conditions from congenital heart defects to vascular malformations. Furthermore, the zebrafish serves as a powerful, integrative platform for modeling a spectrum of diseases and for facilitating both high-throughput drug screening and in-depth mechanistic studies of therapeutic agents.

While challenges remain including anatomical differences, species-specific gene duplication, scaling effects, and high-throughput imaging bottlenecks, the field is experiencing transformative growth. The integration of multi-omics data with patient-specific avatars and AI-driven analytics promises to enhance the translational relevance and predictive power of zebrafish research. By evolving from a robust research tool into a predictive platform for personalized medicine, zebrafish will continue to be at the forefront of cardiovascular science, accelerating the translation of fundamental mechanobiological insights into novel diagnostic and therapeutic strategies for human health.

## Author Contributions

HL: Conceptualization, Writing—Original Draft. KC: The conduct of the work and the collection of the material analysed, Writing—Review & Editing. TG: The conduct of the work and the collection of the material analysed, The organisation, verification and synthesis of the evidence so extracted, Visualization, Writing—Review & Editing. XX: The conduct of the work and the collection of the material analysed, The organisation, verification and synthesis of the evidence so extracted, Writing—Review & Editing. DL: Conceptualization, Supervision, Project Administration, Funding Acquisition, Writing—Review & Editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflicts of Interest

The authors declare no conflicts of interest.

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