GEN Biotechnology
Volume 00, Number 00, 2024

DOI: 10.1089/abc.2024.0020

GEN Biotechnology

REVIEW ARTICLE: DEI IN BIOTECHNOLOGY

Engineered *In Vitro* Models to Improve the Mechanistic Understanding and Treatment of Neglected Tropical Diseases Caused by Protozoan Parasites

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Abstract

Neglected tropical diseases (NTDs), particularly those caused by trypanosomatid protozoa, impose a significant global burden, disproportionately affecting underserved communities in tropical and subtropical regions. Despite their high mortality rates, associated chronic conditions, and rapid spread due to globalization and climate change, NTDs have historically received minimal research investment. Additionally, existing treatments cause severe adverse effects. While animal models have contributed significantly to our understanding of these diseases, they are limited by technical and financial constraints. Current *in vitro* approaches predominantly focus on single-cell interactions on stiff substrates; thus, failing to capture tissue-level dynamics crucial for understanding host-parasite interactions. In this scoping literature review, we summarize emerging engineering applications to address these challenges by developing more complex *in vitro* models. We discuss 36 publications that describe novel strategies employing biomaterials, organoids, spheroids, and microfluidic devices to improve the mechanistic understanding of these NTDs. We also describe how these preclinical models are being used as screening platforms in the drug discovery and repurposing pipeline. To better understand the global scope of this research, we also performed a meta-analysis of the geolocation of the authors whose work was included in this review. This analysis uncovers uneven global participation in these efforts to combat NTDs. Ultimately, we draw attention to the need for a multidisciplinary and transnational approach to mitigate the impact of trypanosomatid NTDs and reduce health inequities globally.

Neglected tropical diseases (NTDs) constitute a diverse group of diseases that disproportionately affect underserved communities around the world, often living in low- and middle-income countries in tropical and subtropical regions.[1] The 2019 Global Burden of Disease Study reported an incidence rate of 58 million cases worldwide, with other estimates suggesting that up to 1 billion people in the world are infected with at least one NTD.[2–4] Despite their significant disease and economic burden, these diseases have garnered limited investment in research and development, and insufficient attention from the biomedical science community. In recognition of this historical neglect, in 2015, the United Nations formally endorsed the inclusion of NTDs as a priority for Sustainable Development Goal 3—"ensure healthy lives and promote wellbeing for all at all ages".[5,6]

Among NTDs, those caused by trypanosomatid protozoa exhibit the highest mortality rates.[2,3] These vector-borne diseases include leishmaniasis, Chagas disease, and human African trypanosomiasis, which result from infection with *Leishmania* spp., *Trypanosoma cruzi*, and *Trypanosoma brucei* ssp., respectively.[7] Because trypanosomatid parasites can persist and establish long-term infections in the host, this group of NTDs not only is the deadliest but also leads to chronic conditions that contribute to long-term disability, impaired quality of life, social stigma, and economic pressure in the communities where they are endemic.[8–11]

The prevalence of trypanosomatid NTDs spans the globe, with these infectious diseases primarily affecting rural populations in Latin America, sub-Saharan Africa, southeast Asia, and the Middle East.[12–14] While NTDs have the greatest impact on impoverished communities in the Global South, the last two decades have seen a sustained increase in the incidence rate of these diseases in upper-middle- and high-income countries.[3] Furthermore, globalization, urbanization, and climate change have expanded the geographical reach of protozoan parasites and their insect vectors from primarily tropical and subtropical regions to previously nonendemic areas.[15] For example, recent estimates indicate a prevalence of ~10,000 cases of locally

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acquired *T. cruzi* infections in the United States, concentrated in southern states and in regions with high numbers of Latin American immigrants.[16,17] Similarly, cutaneous leishmaniasis is now endemic in the United states with researchers at the Center for Disease Control and Prevention recently reporting the identification of a unique strain specific to the country.[18,19] These trends underscore the escalating risk of trypanosomatid NTDs and the importance of developing novel approaches to mitigate their impact on vulnerable populations worldwide.

The World Health Organization's (WHO) 2021–30 road map for NTDs prioritizes key strategic areas that include the development of new diagnostic, vector control, and therapeutic solutions.[20] The integration of innovative biotechnological approaches is crucial to meet these goals. As reviewed elsewhere,[21–23] recent advances in point of care diagnostics such as novel membrane technologies for sample collection and storage, and loop-mediated isothermal amplification are transforming the diagnosis, treatment and surveillance of parasitic NTDs in resource-limited settings.[24–26] In recent years, genetic technologies leveraging the tetracycline repressor system and CRISPR/Cas9 gene editing have also emerged with the goal of suppressing vector populations in endemic areas.[27–29] Despite these advances, significant gaps remain, particularly in the treatment of protozoan NTDs.

The study of these parasites *in vitro* and *in vivo* is challenging due to their complex life cycles across multiple host species, their ability to infect multiple host cell types, and diverse growth conditions. Additionally, current drug treatments for trypanosomatid NTDs present significant drawbacks including high toxicity levels, the development of parasite resistance, limited efficacy in the chronic phase of these infections, and complex administration regimens that lead to poor patient compliance.[11,30] This review examines the literature on emerging biomaterials and tissue engineering applications that aim to improve the study and treatment of leishmaniasis, Chagas disease, and human African trypanosomiasis. First, we present a comprehensive overview of existing strategies to develop more complex *in vitro* models that enable the mechanistic study of these infections and more efficient drug testing. Next, we perform a meta-analysis to assess global participation in this type of research. Finally, we discuss additional opportunities to leverage advances in these biotechnology fields towards the goal of improving treatment outcomes, enhance preventative strategies, and ultimately, reduce the inequities exacerbated by these diseases.

NTDs Caused by Protozoan Parasites

Leishmaniasis

Leishmaniasis is a group of diseases caused by *Leishmania* spp. It is transmitted by various sandflies of the genus *Phlebotomus* in the old world (Asia, Africa, and Europe) and the genus *Luztomya* in the new world (the Americas).[31] Currently, over 98 countries are at risk of infection and disease, with affected areas mostly concentrated in neglected communities in South America, East Africa, South Asia, the Middle East, and the Mediterranean.[14] The presentation of the disease in the human host varies depending on the immune response, with macrophages being the preferred cell type for parasite replication.[32] The disease may be exacerbated by comorbidities such as the coinfection of other pathogens, lack of access to complete nutritional intake, and direct effects of climate change on areas of prevalence of the disease.[33] For example, comorbidity of HIV infected patients that are also infected with *Leishmania* in northeast Brazil has been associated with poor prognosis compared with patients suffering from either disease alone.[34]

The disease presentation in humans (and other vertebrate hosts) is mostly determined by the *Leishmania* species.[35] Depending on the species, the parasites cause different forms of leishmaniasis: cutaneous disease, mucocutaneous disease or visceral disease (Fig. 1). The primary symptoms and complications of these disease presentations are skin lesions, lesions on mucous membranous tissues, and enlargement of internal organs like the liver and spleen, respectively.[36] Although cutaneous and mucocutaneous leishmaniasis have a favorable survivability rate in human patients, visceral leishmaniasis is lethal if left untreated.[35]

Pentavalent antimonials and amphotericin B are currently prescribed as the first line of treatment, with milfetosine, and pentamidine as complementary treatment options.[37] Pentavalent antimonials are often prescribed daily through intravenously or intramuscular administration at 20 mg/kg for 28–30 days. Amphotericin B is currently prescribed intravenously at 0.5–1.0 mg/kg daily or every other day for 28–30 days, for a total of 15–40 mg/kg cumulative drug administration.[37] Miltefosine may be used in conjunction with the aforementioned drugs orally at a concentration of 2.5 mg/kg for 28–30 days with a total dose of 20–60 mg/kg.[38] Antimony and Amphotericin B resistant *Leishmania* strains are rare (<10%), but alternative treatments are very limited in such cases, with poor prognosis for infected individuals.[38] Nonetheless, all of these treatment options present important adverse side effects, including nephro- and hepatotoxicity. Of note, mucocutaneous disease has been reported to be poorly responsive to available antileishmaniasis drugs.[39] Wild and domesticated animals are known to be reservoir hosts of *Leishmania* spp., which further complicates control and containment of the disease due to its zoonotic nature.[31,40]

Chagas disease

Chagas disease, also known as American trypanosomiasis, is caused by *T. cruzi*. It is transmitted to humans through its vector, the triatomine bug.[41] The primary species of the triatomine bug tied with spread of the Chagas disease are *Rhodnius prolixus,* and *Triatoma dimidiate* that defecate while feeding. Chagas is endemic to South and Central America, with cases growing in North America.[12,17] The progression of Chagas disease includes the acute phase, intermediate phase, and chronic phase.[42] The acute phase is characterized by rapid replication and infection of primarily muscle tissue in the human host, with visible symptoms caused by inflammation and necrosis in affected tissues. While the acute phase can usually last between 8 and 12 weeks, chronic infections can remain for years and go undetected due to lack of symptoms.[43] The chronic phase of the disease is characterized by a reduction of parasite burden. However, in some patients, there is hypertrophy primarily in the gastrointestinal tract and heart, where chronic infection can lead to multiple cardiovascular complications including cardiomyopathy,

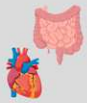
	Leishmaniasis	Chagas Disease	Human Sleeping Sickness
Vector	Sandfly 	Triatomine bugs 	Tsetse fly 
Parasite	*Leishmania* spp.	*Trypanosoma cruzi*	*Trypanosoma brucei* ssp.
Intracellular or Extracellular	Intracellular 	Intracellular 	Extracellular 
Afflicted Organs	• **Visceral:** Liver and spleen • **Cutaneous:** Skin • **Mucocutaneous:** Mucous membranes 	Heart and digestive system 	Lymph nodes and central nervous system 
Initial Symptoms	• **Visceral:** Fever, weight loss, abnormal blood tests, and swollen abdomen • **Cutaneous:** Skin lesions • **Mucocutaneous:** Sores on mucous membranous tissues	Fever, headache, enlarged lymph glands, pallor, muscle pain, difficulty in breathing, swelling, and abdominal or chest pain	Fever, headache, enlarged lymph nodes, joint pains and itching
Chronic Complications	• **Visceral:** Liver and spleen enlargement • **Cutaneous:** Skin lesions • **Mucocutaneous:** Partial or total destruction of mucous membranes	Myocarditis, heart failure, and enlargement of esophagus and colon	Behavior changes, confusion, sensory disturbances and poor coordination, and sleep cycle disturbance

FIG. 1. Key characteristics of protozoal NTDs. NTD, neglected tropical disease.

arrhythmias, myocardial dysfunction, thromboembolic events, apical aneurysms, and, eventually, stroke or sudden cardiac death.[44,45] Current treatments cause frequent adverse events and are ineffective in adult patients who suffer from chronic infection.[46]

The lack of diagnostic tools and high prevalence of asymptomatic infections pose a challenge for the early detection of Chagas disease.[47] Moreover, current treatments are limited to nifurtimox and benznidazole. Nifurtimox is a composition of nitrofurans that were shown to better contain the infection during the acute phase and it is administered orally at a concentration of 8–10 mg/kg per day for roughly 60–90 days.[48] Success rates in acute phase patients ranged between 88–100% in clinical studies of individuals that received a recommended scheduled dose. Chronic intermediate phase infection prognosis was poor as only about 7–8% of patients showed any signs of improvement.[49] Benznidazole treatment is an alternative treatment option for Chagas disease patients that may be sensitive to nifurtimox's stronger side effects. It is recommended to administer benznidazole orally at a concentration of 5–10 mg/kg daily for 30–60 days.[50] Overall effectiveness of the treatment is slightly lower than nifurtimox during the intermediate acute phase at roughly 80% success rate, but the chronic phase also falls short with barely an 8% rate of remission in these patients.[51] The exact cause for the stark discrepancy in prognosis between the acute and chronic phases is yet to be elucidated; however, changes in the lifecycle of the parasite as it enters into the chronic phase of the infection have been long suspected for the loss of effectiveness of the drugs.[47]

Human African trypanosomiasis

Human African trypanosomiasis is caused by two subspecies of *T. brucei* - *T. brucei gambiense* (gHAT), and *T. brucei rhodesiense* (rHAT). The parasite is spread by the *Glossina* fly, also known as tsetse fly.[40] Chronic human African trypanosomiasis can result in daytime somnolence, which has led to the disease condition called sleeping sickness.[52] *T. brucei*, unlike the other kinetoplastid organisms (*Leishmania* spp. and *T. cruzi*) are extracellular parasites in the mammalian host. *T. brucei* species survive in the human host bloodstream by expression of a glycoprotein coat, which allows them to subvert antibodies and the complement system of the host.[53] Human African trypanosomiasis has two stages of disease, the hemolymphatic stage and the meningo-encephalitic stage.[13] The hemolymphatic stage is characterized by the early stages of infection in which the parasite replicates in the lymphatic tissues and bloodstream of the human host. Oftentimes, patients are asymptomatic in this stage, leading to what appears to be a rapid escalation to the meningo-encephalitic stage. This occurs when the parasite crosses the blood–brain barrier, infecting the central nervous system and causing coma or death of the host if left untreated.[13] Therefore, treatments for this second stage of the disease must cross the blood brain barrier.

Treatment of choice depends on the subspecies of *T. brucei* and the stage of the disease. Practical treatments are varied due to the drastically different effects they have on gHAT or bHAT, with bHAT being the most difficult subspecies to treat. Pentamidine has been demonstrated to be quite efficacious

against the first stage of gHAT, as it showed a 93–98% parasite burden reduction in clinical patients with a regime of intravenous administration of 4 mg/kg daily for 7–10 days.[54] However, pentamidine does not cross the blood–brain barrier, making it inapplicable for patients in the second stage of the disease, and it has not been demonstrated to be effective against rHAT.[55] In contrast, fexinidazole has been shown to be effective against gHAT in its first and second stage with a success rate of 99% and 91% respectively.[56] Fexinidazole may be effective against rHAT, and experimental trials are currently underway to demonstrate its efficacy.[57] This drug is preferred over the other treatments in the medical field due to its oral route of administration, as opposed to intravenous or intramuscular administrations.[58]

Combination treatments can also be effective for the treatment of sleeping sickness. Nifurtimox/eflornithine combination therapy can be prescribed to treat the first and second stage of gHAT, with a success rate of >90% in both stages.[59] It is not effective against rHAT, and it is speculated that it may be due to the genetic variances of both subspecies.[60] On the contrary, suramin is effective against both gHAT and bHAT;[61] however, it is limited to the first stage of the disease, since it cannot cross the blood–brain barrier.[62] It is administered intravenously at a dose of 10 mg/kg injections, over the span of 30 days and has a success rate of >80% according to recent medical trials.[63] Fiacoziborole is a relatively new drug effective against gHAT. It was reported to have a 100% success rate in early-stage infections and 92% success rate in second stage infections.[64] The complexity of these treatment regimens underscores the challenges facing the fight against all forms of human African trypanosomiasis.

Limitations of existing *in vitro* and *in vivo* models

Although animal models have widely contributed to our understanding of host–parasite interactions and response to infection, identifying biological mechanisms in these systems is often challenging, requiring large technological, financial, and time investments (Fig. 2).[65] Traditional *in vitro* approaches to study Chagas disease, leishmaniasis and African sleeping sickness either study trypanosomatid parasites in isolation or focus on studying interactions between these microorganisms and a single-cell type, usually macrophages or other immune cells (Fig. 2). Protozoan infections are frequently chronic, causing organ enlargement (organomegaly) driven by parasite invasion and proliferation within specific tissues.[66] Yet, there is limited understanding of the molecular factors and cellular interactions that drive tissue, vascular, and extracellular matrix (ECM) remodeling in parasitic infections. Current studies are usually performed on tissue culture polystyrene (TCPS), a stiff substrate devoid of physiologically relevant physical and biochemical cues. Such single-cell approaches ignore interactions between multiple cells in tissues and the contributions of the ECM, features that are crucial for tissue homeostasis and parasite persistence.[67,68] The limitations of current models make it difficult to accurately delineate parasite behavior in *in vivo* conditions, and to develop effective diagnostic and pharmacological tools for the chronic complications of protozoal NTDs (Fig. 2). Thus, there is a critical need for physiologically relevant *in vitro* infection models that capture multicellular interactions and incorporate the properties of native human tissues.

Engineering Improved *In Vitro* Models of Protozoal NTDs

Tissue engineering-based *in vitro* approaches have recently emerged as promising alternatives to traditional models of parasitic infections (Fig. 2).[69] Utilizing advanced technology such as biomaterial scaffolds, microfluidic devices, and organoids, researchers create tissue mimics that accurately reproduce the

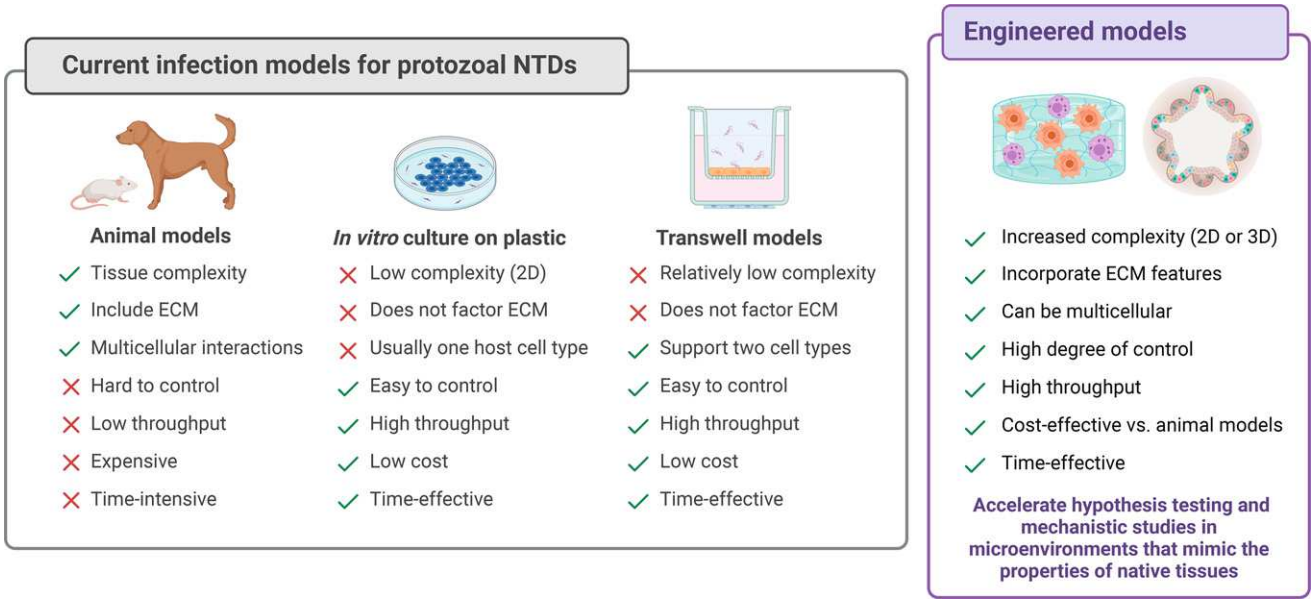


FIG. 2. Advantages and limitations of the most widely used experimental models to study protozal NTDs.

cellular architecture and biomechanical properties of human organs.[70] Compared with animal models, these engineered tissue constructs are highly controllable and time- and cost-effective.[71] By independently modulating mechanical and biochemical cues (e.g., stiffness, ECM composition, and flow rates), researchers can precisely design microenvironments that mimic features of native and diseased tissues while supporting the physiological functions of both host and parasite cells.[72–74] These models can also support the growth of multiple human-derived cell types in both 2D and 3D, increasing the capacity to mimic the complex cellular interactions within host tissues.[71] The integration of tissue engineering with disease modeling enhances the ability to study host–parasite interactions and tissue-level phenomena, like ECM remodeling, that are pivotal in the progression of parasitic infections. Thus, these sophisticated *in vitro* infection models can accelerate the process of hypothesis testing, thereby leading to the identification of disease and infection mechanisms relevant to human health.

In this review, we sought to explore biomaterials, and cell and tissue engineering strategies that have been applied for the development of more complex *in vitro* models of protozoal NTDs. To identify relevant publications, we conducted a thorough literature search in four databases: PubMed, Web of Science, Scopus, and Embase with no specified start date and an end date of July 2, 2024 (Fig. 3). First, we used keywords related to the three protozoal parasitic NTDs recognized by the WHO (Chagas disease, leishmaniasis, and trypanosomiasis). Next, we supplemented our search with keywords associated with complex *in vitro* models including cell and tissue engineering, organoids, microfluidics, microphysiological systems, and biomaterials. The final list of keywords employed for each database and step-by-step results for each search strategy can be found in Supplementary Table S1. A total of 1,618 publications were identified across all databases. After removing publications found in more than one database and excluding papers classified by these databases as reviews or conference abstracts, the literature search was narrowed down to 689 publications for further screening (Fig. 3). The title, abstract, and full text of these publications were then manually screened by two members of the research team using Covidence software.

All publications marked for inclusion met the following criteria: (1) must be a primary research article, (2) must focus on at least one of the three protozoal NTDs, and (3) must use engineered *in vitro* models of the types described in the key words. A total of 653 publications were manually excluded by the research team (44 unrelated to protozoal NTDs, 44 were not primary research articles, and 565 did not employ the engineered *in vitro* models described in our key words). This screening process concluded with the identification of 36 relevant publications (Fig. 3).

The 36 publications included in this scoping review introduce novel approaches to model protozoal infections that leverage natural biomaterials, spheroids, organoids, synthetic hydrogels, and microfluidic devices for the development of

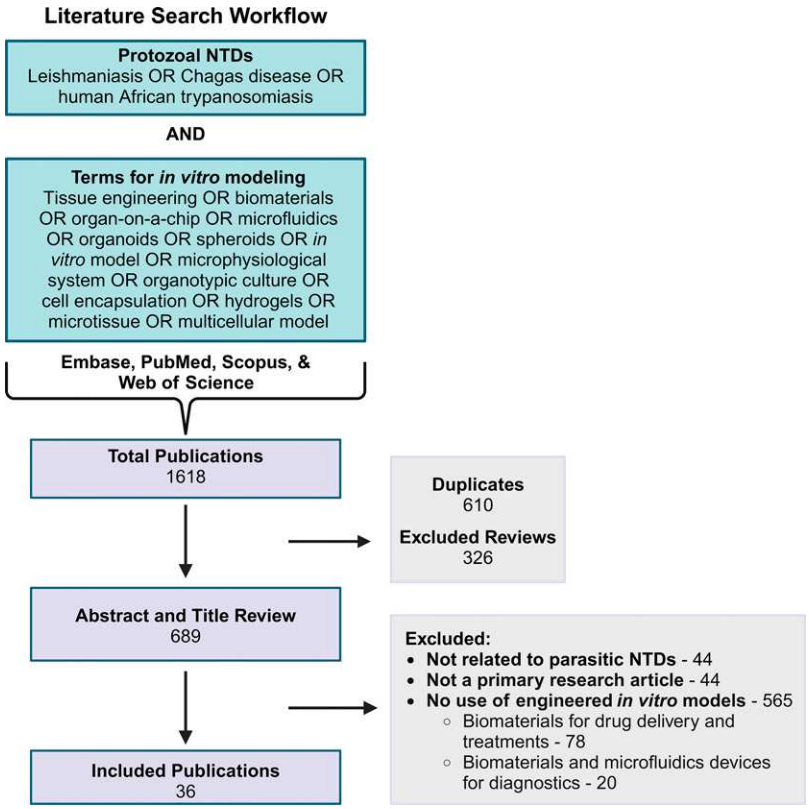


FIG. 3. Overview of the methodology employed for our scoping literature search.

more physiologically relevant *in vitro* infection models that better capture the complexity of human physiology and host–parasite interactions compared with existing models (Fig. 4). These strategies can be classified in two broad categories: models to study host–parasite interactions, and solutions to improve the study of parasite biology in the absence of host cells. Here, we describe how researchers are using these technologies to both increase mechanistic understanding of protozoal NTDs, and screen novel treatment strategies for these diseases.

Developing Models to Study Host–Parasite Interactions *In Vitro* and *Ex Vivo*

Natural biomaterials to create more physiologically relevant infection models

One strategy to overcome the limitations of culture on stiff tissue culture plastic is the use of natural biomaterials derived from animal ECM proteins. These natural biomaterials provide both mechanical and biochemical cues that can guide cell behavior and modulate host responses to infection. Collagen, in particular, has emerged as an attractive alternative for the study of trypanosomatid infections due to its relatively low cost, ease of use, and high abundance in many of the tissues targeted by these parasites (e.g., heart, liver, intestines).[67,68] The earliest use of collagen in a more complex model identified in our literature review consists of collagen coatings to generate an *in vitro* model of the blood–brain barrier for the study of central nervous system invasion by *T. brucei*.[75] To create the model, Grab et al. cultured monolayers of primary human brain microvascular endothelial cells on transwell inserts coated with type I collagen. The inclusion of collagen increases cell adhesion and stimulates the development of a continuous epithelial barrier.[76] Using this model, human infective *T. brucei gambiense* strains were found to decrease endothelial membrane integrity and traverse the barrier paracellularly.[75] In comparison, no changes in transepithelial integrity or migration were observed in animal infective *T. brucei brucei* strains.

Hydrogels derived from decellularized tissues have also been used as coatings for *in vitro* modeling of protozoal NTDs.[77,78] Compared with collagen coatings, these gels encompass a broader spectrum of components, providing a rich environment that closely resembles the native ECM.[79] Two of the most widely used ECM hydrogels for *in vitro* application are Matrigel and Geltrex, commercially available basement membrane extracts derived from decellularized murine tumors.[80] These[80] products contain collagen IV, laminin, and additional growth factors that support cell adhesion and function, specially for primary cells, stem cells, and other cells that are difficult to culture on TCPS alone. In 2018, da Silva Lara demonstrated that cardiomyocytes derived from human induced pluripotent stem cells cultured on Geltrex could reproduce the intracellular cycle of *T. cruzi* after infection.[77] In this model, *T. cruzi* parasites successfully responded to treatment with benznidazole, the current standard of care for Chagas disease—demonstrating the utility of this approach for future secondary screening applications. Similarly, de Almeida-Leite used Matrigel to culture primary neurons isolated from sympathetic cervical ganglia that were later cocultured with *T. cruzi*-infected macrophages.[78] This coculture system allowed the researchers to established that while neurons did not respond to the parasites alone, nitric oxide released by infected macrophages could induce neuronal damage.

Natural biomaterials can also be manufactured as gels for the embedding of cells in three-dimensional culture. These hydrogel matrices provide biochemical and mechanical cues that resemble the properties of native ECM.[72] Logullo et al., for instance, used this approach to understand the impact of macrophage-collagen interactions on *T. cruzi* infection.[81] Primary mouse peritoneal macrophages were cultured on TCPS, TCPS coated with collagen type I, or plated on top of a 3D collagen I gel. The authors observed marked differences in macrophage responses to the infection depending on culture method, with the gel leading to the early release of trypomastigotes. Compared with culture on TCPS and the collagen coating, macrophage culture on collagen hydrogels also led to higher secretion of proinflammatory and profibrotic cytokines, and a more migratory cell morphology in response to the infection. These results demonstrate that both the identity and presentation of the culture substrate affects the cellular response to infection. In a similar approach, Luz et al. embedded macrophages and dendritic cells in a collagen I gel to study cell migration after infection with different *Leishmania* species.[82] For the macrophages, regardless of *Leishmania* species, infection resulted in reduced migration. In contrast, the dendritic cells exhibited decreased, unaffected, or enhanced migration compared with the control depending on the parasite species. Observing these interesting cell-type specific behaviors would be difficult in two-dimensional culture where cellular movement is limited.

Natural biomaterials can also be used to study how parasites migrate and interact with ECM in the absence of host cells. Petropolis et al. embedded *L. amazonensis* parasites in a collagen I gel.[83] The researchers were then able to quantify the release of both metallo- and cysteine proteases that remodeled the gel, demonstrating that *Leishmania* parasites can interact directly with ECM proteins. Inhibition of these proteases led to a reduction in promastigote invasion. These results suggest that *Leishmania* protozoa actively interact with host ECM and degrade it to facilitate migration.

Collectively, these seminal studies using ECM-derived hydrogels showcase the advantages of biomaterials-based culture platforms that enable the observation of phenomena in three dimensions and the study of host–parasite interactions at the tissue level.

Spheroids and organoids to study host–parasite interactions

Spheroids, free floating aggregates of cells, have emerged throughout the last decade as an alternative to culture on plastic that enables the study of cellular interactions in three dimensions.[84,85] The three-dimensional organization of these cells and increased cell-cell contact results in gene expression patterns and cell behavior that more closely mimics *in vivo* scenarios than traditional culture approaches on plastic.[86,87] In the context of parasitic NTDs, spheroids have primarily been employed

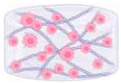
	Approach	Advantages	Limitations	References
Host-parasite interactions	**Natural Biomaterials** 	• Capture 3D cell behavior • Mimic cell- and protozoan-ECM interactions • Enable the study of parasite and host cell migration • Control of microenvironmental properties (e.g. stiffness, composition) • Relatively simple to use, depending on the material and source	• Increased costs compared to 2D culture • Batch-to-batch variability • Limited mechanical and biochemical tuning depending on the material • If used in 3D, require confocal microscope for visualization • Reductionist approach – do not capture all structural and compositional cues of the ECM	Grab, *J Parasitol* (2004)[75]; da Silva Lara, *Microbes Infect,* (2018)[77]; de Almeida-Leite, *Neurobiol Dis* (2007)[78]; Logullo, *Life Basel Switz* (2023)[81]; Luz, *Front Cell Dev Biol* (2023)[82]; Petropolis, *PeerJ* (2014)[83]
	Spheroids and Organoids 	• Capture 3D cell behavior • Useful to study parenchymal cell responses to infection • Can contain multiple cell types • Suitable for culture of primary cells and tissues • Preserve cellular organization of native tissue • Can study infection of multiple cell types simultaneously	• Expensive compared to 2D culture • High batch-to-batch variability • Specialized expertise required to execute organoid protocols • Organoid differentiation/assembly protocols can be time consuming • Lumen can be difficult to access • Require confocal microscope for visualization	Rodriquez, *Biomedicines* (2020)[88]; Silberstein, *Front Microbiol* (2021)[89]; Silberstein, *Front Microbiol* (2023)[90]; Garzoni, *J Infect Dis* (2008)[93]; Nisimura, *MOl Biochem Parasitol* (2020)[94]; Ferrão, *Exp Cell Res* (2018)[95]; Orlando, *Molecules* (2021)[98]; Orlando, *Biology* (2023)[99] ; Chandrasegaran, *F1000Research* (2023)[103]; Daghero, *Front Cell Infect Microbiol* (2023)[104]
	Organotypic and Explant Organ Cultures 	• Capture 3D cell behavior • Useful to study parenchymal cell responses to infection • Increased complexity • Contain all cell types and ECM components • Preserve cellular and ECM organization of native tissue	• Prone to contamination • Highly variable • Requires animal experiments or clinical sample • Poor control of microenvironmental variables	Stoppini, *Int J Med Microbiol* (2000)[107]; Postan, *Int Arch Allergy Immunol* (2009)[108]; Tanowitz, *Am J Trop Med Hyg* (1982)[109]; McCabe, *Exp Parasitol* (1989)[110]
Parasite Biology	**Synthetic hydrogels** 	• Enhanced single cell characterization • Does not require parasite fixation • Can study parasite motility • Easily combined with high resolution live-cell imaging	• Do not incorporate fluid mechanics and dynamics • Complex trapping • Require confocal microscope for visualization • "Matrix" might not resemble the properties of the ECM • Not as high throughput as 2D or liquid culture	Glogger, *Exp Parasitol* (2017)[112]; Dong, *PLOS ONE* (2018)[113]
	Microfluidic Devices 	• Enhanced single cell characterization • Does not require parasite fixation • Mimic fluid mechanics and dynamics • Can study parasite motility • Easily combined with high resolution live-cell imaging	• Complex fabrication process (although some devices are commercially available) • Require expertise that limits accessibility • Not as high throughput as 2D or liquid culture	Oldenburg, *Sci Rep* (2021)[115]; De Niz, *PloS One* (2023)[116]; Uppaluri, *Biophys J* (2012)[117]; Stellamanns, *Sci Rep* 2014[118]; Hochstetter, *Lab Chip* (2015)[119]; Cadena, *Microchem K* (2024)[120]; Vargas Jiménez, *Ultrasound Med Biol* (2022)[121]; Voyton, *Biochemistry (*2019)[123]

FIG. 4. Summary of current approaches to engineer improved *in vitro* and *ex vivo* models to study host–parasite interactions and parasite behavior.

to explore the impact of infection on parenchymal cell behavior, with marked differences observed between these approaches and traditional 2D culture.[88–90] Using primary canine liver cells, Rodrigues et al. were able to demonstrate that hepatocyte spheroids generate an innate immune response to *L. infantum* with higher levels of nitric oxide production observed in spheroids compared with culture on TCPS.[88] In a similar study, Silberstein et al. modeled the placental barrier using spheroids formed from a trophoblast-derived cell line and human brain microvascular endothelial cells to study the mechanisms of mother-to child transmission in Chagas disease.[89,90] Unlike trophoblasts grown in 2D, the syncytiotrophoblast spheroids were resistant to *T. cruzi* infection. Furthermore, the infected spheroids released paracrine factors that prevented *T. cruzi* infection of other nontrophoblastic cells.[89] These findings demonstrate the potential of spheroid culture models to uncover the mechanisms that govern host–parasite interactions.

Similar to models that employ natural materials, spheroids allow the study of three-dimensional phenomena like parasite transmigration. After infecting HeLa spheroids, a 2020 study found

differences in invasiveness, migration, and infection rates between different *T. cruzi* strains.[91,92] Known virulent strains were highly invasive and able to transmigrate deeply into spheroids, while poorly virulent strains remained in the external layers. Moreover, clinical *T. cruzi* strains isolated from congenitally infected children exhibited a highly migratory phenotype in the spheroids in contrast with an isolate from an infected mother that did not transmit the infection to her children.[91] This study emphasizes the ability of spheroid models to replicate clinically relevant phenomena.

As described earlier in this review, *T. cruzi* infections primarily target the heart and are associated with chronic cardiovascular complications.[45] Due to the difficulty in deriving and expanding cardiomyocytes in 2D culture,[87] cardiac spheroids have gained popularity as model systems for Chagas disease research and drug screening.[93,94] In 2008, Garzoni et al. demonstrated that *T. cruzi can* successfully invade cardiac spheroids derived from mouse embryos.[93] These cardiac spheroids responded to the infection by depositing more ECM, a behavior directly linked to the fibrotic response usually observed clinically during Chagas disease. Using these spheroids, the team identified that inhibition of transforming growth factor beta, a profibrotic cytokine, led to reduced ECM deposition and a decrease in parasite load.[95] Having demonstrated the utility of their cardiac spheroids for the study of fibrogenesis, the Garzoni research group then used them to evaluate the antiparasitic efficacy of posaconazole, an antifungal treatment.[94] Treatment with posaconazole led to a 50% decrease in parasite load and ECM production. However, the latest clinical trials with this medication did not sustain *T. cruzi* clearance in humans.[96,97]

Spheroids can also be combined with computational approaches to further optimize the drug discovery pipeline. In 2021, Orlando et al. reported the use of rational drug design in combination with a 3D spheroid model to optimize the screening of pyrazole derivatives.[98] *In silico*, the team first generated 44 analogs based on a hit compound targeting cruzipain, a key *T. cruzi* enzyme involved in evasion of the host immune system, invasion, and intracellular replication. Three of the screened compounds exhibited promising trypanocidal activity, including significantly reduced viability in 3D cardiac spheroids generated from murine heart muscle cells.[99] Further work will be necessary to establish the efficacy and safety of these candidates *in vivo;* nonetheless, this study demonstrates the potential of engineered *in vitro* models as predictive preclinical platforms.

While spheroids represent an important evolution for *in vitro* infection models, they still exhibit an important limitation: the inclusion of only one or two cell types. In contrast, organoids include more diverse cell types native to the tissue of interest, while preserving crucial architectural properties such as apical and luminal polarization.[100,101] Organoids are made by culturing a small piece of tissue or an aggregate of stem cells in the presence of the desired tissue's growth factors.[102] The use of organoids for the study of protozoal NTDs is still in its nascent stages. In 2023, Chandrasegaran et al. reported the development of a 3D neural model of human African sleeping disease using induced pluripotent stem cells stimulated with neural induction media.[103] The authors demonstrated that the organoids are able to sense the presence of *T. brucei* and respond in the absence of immune cells by upregulating genes related to immune cytokines, monocyte recruitment, and angiogenesis.[103] In another proof of concept study, Daghero et al. created both murine and human colon-derived organoids as intestinal models of *T. cruzi* infection.[104] In both cases, parasites were observed within phagocytic and nonphagocytic cells, penetrating from the basolateral and apical sides of the organoid.[104] However, only some cell types were infected by the parasites. Even though further research is necessary to understand the observed cellular tropism, this highlights the importance of including multiple cell types to understand host–parasite interactions at the tissue level.[104]

Ex vivo culture systems that preserve tissue integrity

Hydrogel and spheroid *in vitro* platforms offer high degrees of flexibility and control to generate microenvironments for studying specific cell behaviors and cell–ECM interactions in response to infections. However, these models sometimes lack the structural complexity of biological tissues. In contrast, *ex vivo* culture systems that sustain organ slices or whole organs in culture preserve not only multicellular complexity but also organ-specific architectures critical for physiological function (Fig. 4).[105] Organotypic culture systems consist of thin slices of organs that can be maintained with culture media for up to several days or even weeks.[106] The ability to culture these systems for prolonged periods of time is particularly attractive of prolonged parasitic infections. For example, Stoppini et al. investigated the consequence of *T. brucei* infection on central nervous system tissue, using cultured neonatal rat hippocampal slices.[107] Using this system, they authors were able to establish that, while most of the trypanosomes localize to peripheral areas of the tissue, many of them also penetrate deeper even invading glial cells and astrocytes. Because the tissue slices can survive for several weeks, this model can mimic the late stages of human African trypanosomiasis.

Explant organ cultures consisting of whole organs or large pieces of the organ retain an even higher degree of complexity. Explant organ cultures have been used extensively to study Chagas disease due to the diverse tissue tropism exhibited by *T. cruzi* parasites.[108–110] As early as 1981, Tanowitz et al. evaluated parasite-neuronal interactions using cultured murine neonatal spinal cords and dorsal root ganglia.[109] Though neurons were rarely parasitized, dendrites swelled, and axons lost their morphology after infection, demonstrating the utility of these platforms to unravel cell-specific responses to infection. Rather than infecting *ex vivo*, other groups have established explant cultures using organs isolated from mice chronically infected with *T. cruzi*. With this approach, Postan et al. were able to isolate mast cells that formed on the infected hearts during the first week of *ex vivo* culture.[108] These cells were found primarily in fibrotic areas. These studies exemplify the potential of *ex vivo* culture systems that can further bridge the gap between *in vitro* and *in vivo* infection models.

Designing Solutions to Improve the Study of Parasite Biology

Synthetic hydrogels to immobilize parasites for high-resolution imaging

The ability to visualize parasite behavior in response to stimuli is crucial to understand the etiology of protozoal NTDs. Given the size of these microorganisms, high-resolution imaging is necessary to resolve the cellular structures and movement patterns of these microorganisms. However, the highly mobile nature of trypanosomes, and particularly of *T. brucei sspp.,* is a major hurdle for their visualization.[111] A common solution to this problem is the use of chemical fixatives that result in cell death. This approach restricts the application of live high-resolution imaging techniques that study dynamic responses of the parasites over time. Synthetic hydrogels are emerging as alternatives that can physically immobilize the trypanosomatid parasites without compromising imaging resolution.[112,113] For example, Glogger et al. synthesized poly(ethylene glycol) hydrogels functionalized with either norbornene or thiol moieties for UV-induced photocrosslinking.[112] Because the hydrogel matrix can physically contain the parasites, the plasma membranes of *T. brucei* embedded in these gels could be studied using fluorescence super-resolution microscopy. Nonetheless, the hydrogels could only maintain viability for up to 1 h, limiting the time scale of the studies. To address this issue, Dong et al. designed a thermogelling gel-microbead matrix consisting of Pluronic F127 mixed with polystyrene microbeads.[113] Because Pluronic F127 undergoes gelation only under specific temperatures, immobilization of the microorganisms is reversible through modulation of temperature. In this system, *T. brucei* parasites could be reversibly immobilized for high-resolution imaging of thrashing patterns without observing cell death. These studies show that hydrogels are a viable option for live, high-resolution imaging of exceptionally mobile protozoa, with the potential of immobilization to be reversed, enabling longitudinal observation.

Microfluidic devices to explore parasite behavior and physiology

Leishmaniasis and Chagas disease are caused by intracellular protozoa and, as a result, *in vitro* models of these infections are centered on understanding host cell responses to these parasites. In contrast, the *T. brucei* ssp. responsible for African sleeping sickness are highly motile extracellular parasites that can circulate in the bloodstream and inhabit interstitial tissue spaces.[114] Classical methods limit the study of these free-swimming parasites because they involve parasite fixation and lack physiological stimuli such as blood flow. Additionally, many approaches are unable to provide data at single-cell resolution. Microfluidic devices have the potential to overcome these limitations and improve upon existing large scale culture methods. For example, Oldenburg *et. al* used droplet microfluidics to isolate *T. brucei* parasites in emulsion drops that enable the study of parasite variants (including those with slow dividing rates) with single-cell resolution.[115] Moreover, the droplets acted as minibioreactors that sustained parasite growth and expansion over several days, yielding trypanosome titers that exceeded those of standard bulk cultures.

Microfluidic devices also serve as valuable tools for characterizing parasite motility. Due to their high swimming speed and wide range of motion, live imaging of *T. brucei* has proven challenging without partial or total parasite immobilization. To address this challenge, De Niz et al. designed polydimethylsiloxane (PDMS) microfluidic traps to spatially confine *T. brucei* parasites.[116] By optimizing trap height, geometry, and density, the researchers were able to reliably restrict the parasites for longitudinal imaging for up to 8 h without compromising parasite flagellar motility. PDMS microfluidic devices have also been used to study *T. brucei* self-propulsion in a range of physiologically relevant flow conditions.[117] Stellamanns et al. arrived at a different solution by employing optical tweezers to trap living trypanosomes within a microfluidic device for a few minutes with the goal of studying mobility patterns in *T. brucei* in real time.[118] The research team later combined this approach with chemical gradients to engineer a device containing microchambers where *T. brucei* parasites are exposed to trypanocidal compounds.[119] The researchers used this device to investigate the effect of 2-deoxy-D-glucose and glutaraldehyde on parasite motility. Others have also fabricated serial dilution generators to screen experimental compounds against *T. cruzi*, highlighting the potential of these devices for drug discovery.[120] In both cases, the devices allowed for precise control of drug concentrations, and simultaneous high-resolution single-cell imaging.

The versatility of microfluidic devices enables their combination with other types of technology. For example, Vargas Jiménez et al. exposed *Leishmania* parasites to ultrasonic standing waves in a microfluidic device.[121] In this proof-of-concept study, the team reported that both amastigotes and promastigotes respond to acoustic stimulation demonstrating the applicability of ultrasound technology for the noninvasive manipulation of trypanosomes.

Despite their many advantages, an important limitation of most microfluidic devices is their relatively complex fabrication process.[122] Commercially available, prefabricated microfluidic devices can address this limitation and broaden access to this type of technology for all researchers. A 2019 study demonstrated that it is possible to repurpose the CellASIC ONIX microfluidic system originally designed for bacteria for the culture of *T. brucei* parasites.[123] This system enabled the perfusion of the culture medium with glucose at various concentrations and simultaneous live single-cell imaging to assess the response of the parasite. This type of approach provides an alternative for those unfamiliar with microfluidic fabrication.

The studies summarized here demonstrate the potential of microfluidic devices for a wide variety of applications, including the study of parasite motility, high-throughput drug screening, and parasite behavior upon chemical and mechanical stimulation.

Mapping the Global Distribution of Protozoal NTD Research

Despite the global impact of NTDs, research contributions and participation remain unevenly distributed, underscoring significant inequities that reflect both the geographic and economic

disparities associated with these diseases.[124,125] To better understand the global scope of the publications identified in this systematic review, we performed an analysis on the geographic distribution of the authors whose papers met all inclusion criteria. For this analysis, Web of Science citation records for all 36 publications were downloaded and processed using the R refsplitr package.[126] Briefly, author names and affiliations were extracted for each publication yielding a total of 221 identified authors. Author disambiguation was performed to ensure no authors were counted more than once. Based on affiliation information, author location was georeferenced to match the country where the authors were located as reported in each publication (Fig. 5).

We identified 85 authors from a total of 12 countries, all located within the western hemisphere (Fig. 5A). Latin America and the Caribbean was the region with the highest concentration of authors, with the highest representation of authors observed at institutions located in Brazil and the remaining Latin American authors working in South America (Fig. 5). This is partially to be expected considering that two of these parasitic NTDs (Chagas Disease and leishmaniasis) are endemic to this area of the world. Nonetheless, no authors were identified in the Middle East, South and East Asia, and Sub-Saharan Africa despite experiencing significant healthcare and economic burdens from these diseases (Fig. 5B). Outside of Latin America, only the regions of Europe & Central Asia, and North America had researchers authoring the work described here (Fig. 5B). It is important to note that the literature searches prioritized publications written in English and might therefore ignore publications written in other knowledges by authors outside of the regions identified here. Nevertheless, these results describe uneven participation in research related to the development of preclinical NTD *in vitro* models across the world.

Future Opportunities and Outlook

Solving the complicated challenges that contribute to the disparities associated with parasitic NTDs requires multidisciplinary perspectives. The 36 groundbreaking studies described earlier apply engineering and materials science principles to the study of parasitic infections in microenvironments that more closely mimic *in vivo* conditions. These models were used to uncover mechanisms of migration, infection, and trypanocidal activity that would not be possible to study in traditional culture systems. Nonetheless, the small number of papers identified highlights the current reliance on traditional *in vivo* and *in vitro* models for the study of NTDs. Biomaterial scientists and tissue engineers are uniquely positioned to address this challenge by developing more accurate disease models that advance our understanding of these diseases, and contribute to the design of innovative treatments. These interdisciplinary collaborations have the potential to transform the fight against protozoan parasites that perpetuate cycles of poverty and inequality.

Emerging engineering technologies can further contribute to the development of increasingly more physiologically relevant disease models. For example, both natural and synthetic biomaterials can be designed to independently control stiffness and ECM composition, enabling the simultaneous modulation of multiple microenvironmental properties.[73,74,127,128] The introduction of technologies such as reversible hydrogel crosslinkers and pneumatic valves has also enabled the dynamic control of experimental variables in both biomaterials and microfluidic platforms.[128,129] Because tissue remodeling is common during prolonged parasitic infections, leveraging these modular systems to simulate diseased microenvironments over time could allow researchers to uncover previously unknown parasite behaviors specific to the chronic stages of these NTDs. Similarly, advances in 3D printing, organs-

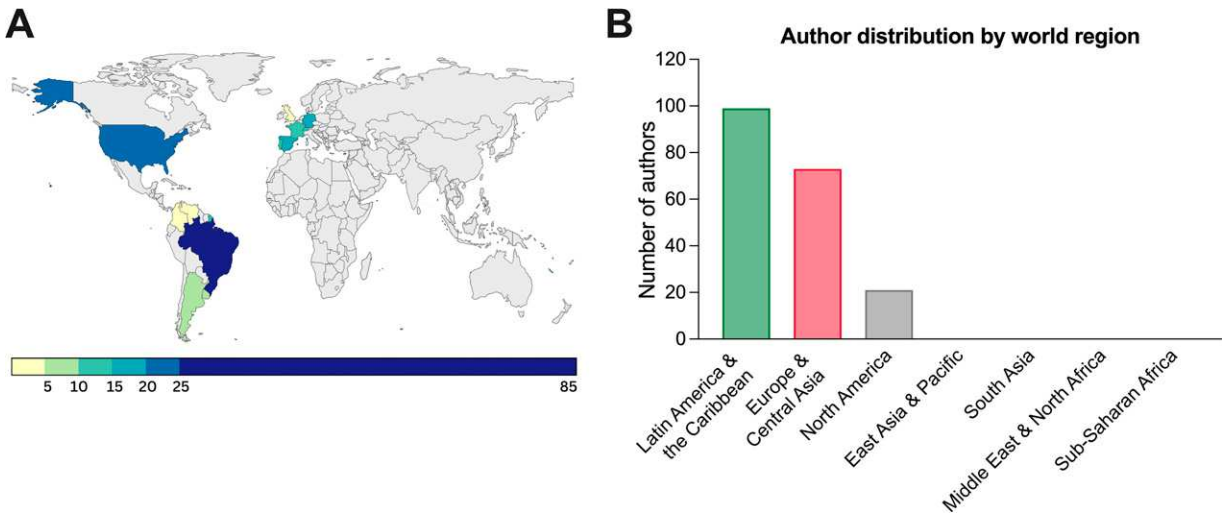


FIG. 5. Geographic distribution of the researchers who authored the publications identified in this systematic review.
(A) Global heat map of author geographical affiliations. Note the nonlinear scale of this heat map. Gray countries were not represented in this dataset.
(B) Histogram of the number of authors represented in this dataset grouped by the world region based on the location of their institutional affiliations. Regions with no bars did not have any authors represented in this dataset.

on-a-chip, and organoid technology have led to the development of increasingly sophisticated tissue and organ models.[102,130–132] Adapting these existing systems for the study of neglected parasitic infections would represent a significant leap forward for *in vitro* modeling of these diseases.

The current medications approved for the treatment of protozoal NTDs face significant challenges, including limited efficacy, high toxicity due to broad systemic effects, low patient compliance, and the development of drug resistance.[30,37,133] Given the significant drawbacks of existing treatments, the WHO is currently prioritizing the development of effective, safe, and affordable treatment interventions in their current strategic roadmap to end NTDs.[20] The generation of more complex *in vitro* models can play a crucial role in addressing this dire need. In 2022, the Food and Drug Administration Modernization Act 2.0 was introduced in the United States to allow the use of organoids, organs-on-chips, cell-based assays, and other engineered *in vitro* models to replace animal testing for the study of drug safety and effectiveness.[134] Similar efforts are gaining traction in other parts of the world.[135] As illustrated by several of the examples highlighted here,[77,94,98] combining preclinical *in vitro* models with novel drug delivery and treatment approaches could revolutionize the development pipeline for new therapeutic strategies by disqualifying nonviable formulations and identifying promising candidates faster.

Our meta-analysis of global participation in this interdisciplinary field revealed high participation by researchers primarily based in Latin America and Europe, with no authors identified in other regions of the world where protozoal NTDs are endemic (Fig. 5). These observations highlight the importance of continuing to build capacity and increase financial support for the researchers pioneering biomaterials and tissue engineering applications to NTDs in the Global South. Considering European, North American, and East Asian researchers have traditionally led the development of cutting-edge biomedical engineering technology, international collaborations between the Global North and South could help address uneven global participation in the application of this field to protozoal NTDs. These collaborations should be rooted in equitable practices that acknowledge local expertise, avoid helicopter science, and respect differences in cultural norms and capacities.[136–138]

Ultimately, the fight against NTDs will require not only scientific and technological advancements, but also global cooperation and an understanding of the socio-economic factors involved, as well as systems-level interventional approaches that consider local health systems, institutional commitment, and community needs.[9,139] This comprehensive approach will not only combat these parasitic diseases but also contribute to the broader goal of achieving global health equity.

Acknowledgments

We would like to acknowledge Samanvitha Deepthi Sudi and Karen Mancera Azamar for their constructive and meaningful feedback on the figures included in this article. Icons for the figures were generated in BioRender©.

Authors' Contributions

T.M. summarized a majority of the studies included in this review and participated in the production of Figures 1–4. J.R. summarized protozoal NTDs. J.A.R. summarized microfluidic device approaches. A.J.H. created Figure 5. P.E.K. provided technical advice and edited the article. A.M.P. developed the idea for the article, generated the ideas for all of the figures, supervised the team, edited, and finalized the article.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

T.M. was supported by a Graduate Research Fellowship from the National Science Foundation (NSF; Grant No. DGE-2236414). J.A.R. was supported by the MARC GatorSTAR program funded by the National Institute of General Medicine at the National Institute of Health (Grant No. T34GM118272). A.M.P. was supported by the NSF Faculty Early Career Development Program (Grant No. 2338708). J.R. was supported by the Latin American and Caribbean Scholarship from the University of Florida.

Supplementary Material

Supplementary Table S1

References

1. Mitra AK, Mawson AR. Neglected tropical diseases: Epidemiology and global burden. Trop Med Infect Dis 2017;2(3):36; doi: 10.3390/tropicalmed2030036
2. Hotez PJ, Alvarado M, Basáñez M-G, et al. The global burden of disease study 2010: Interpretation and implications for the neglected tropical diseases. PLoS Negl Trop Dis 2014;8(7):e2865; doi: 10.1371/journal.pntd.0002865
3. Lin Y, Fang K, Zheng Y, et al. Global burden and trends of neglected tropical diseases from 1990 to 2019. J Travel Med 2022;29(3):taac031; doi: 10.1093/jtm/taac031
4. Hotez PJ, Molyneux David H, Fenwick A, et al. Control of neglected tropical diseases. N Engl J Med 2007;357(10):1018–1027; doi: 10.1056/NEJMra064142
5. Fitzpatrick C, Engels D. Leaving no one behind: A neglected tropical disease indicator and tracers for the sustainable development goals. Int Health 2016;8 Suppl 1(Suppl 1):i15–i18; doi: 10.1093/inthealth/ihw002
6. United Nations. Transforming our world: The 2030 agenda for sustainable development. 2015.
7. Feasey N, Wansbrough-Jones M, Mabey DCW, et al. Neglected tropical diseases. Br Med Bull 2010;93(1):179–200; doi: 10.1093/bmb/ldp046
8. Hotez PJ, Fenwick A, Savioli L, et al. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 2009;373(9674):1570–1575; doi: 10.1016/S0140-6736(09)60233-6
9. Conteh L, Engels T, Molyneux DH. Socioeconomic aspects of neglected tropical diseases. Lancet 2010;375(9710):239–247; doi: 10.1016/S0140-6736(09)61422-7
10. Weiss MG. Stigma and the social burden of neglected tropical diseases. PLoS Negl Trop Dis 2008;2(5):e237; doi: 10.1371/journal.pntd.0000237
11. Varikuti S, Jha BK, Volpedo G, et al. Host-directed drug therapies for neglected tropical diseases caused by protozoan parasites. Front Microbiol 2018;9:2655.
12. Gómez-Ochoa SA, Rojas LZ, Echeverría LE, et al. Global, regional, and national trends of chagas disease from 1990 to 2019: Comprehensive analysis of the Global Burden of Disease Study. Glob Heart 2022;17(1):59; doi: 10.5334/gh.1150
13. Büscher P, Cecchi G, Jamonneau V, et al. Human African trypanosomiasis. Lancet 2017;390(10110):2397–2409; doi: 10.1016/S0140-6736(17)31510-6
14. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012;7(5):e35671; doi: 10.1371/journal.pone.0035671

15. Tidman R, Abela-Ridder B, de Castañeda RR. The impact of climate change on neglected tropical diseases: A systematic review. Trans R Soc Trop Med Hyg 2021;115(2):147–168; doi: 10.1093/trstmh/traa192
16. Irish A, Whitman JD, Clark EH, et al. Updated estimates and mapping for prevalence of chagas disease among adults, United States. Emerg Infect Dis 2022;28(7):1313–1320; doi: 10.3201/eid2807.212221.
17. Bern C, Messenger LA, Whitman JD, et al. Chagas disease in the United States: A public health approach. Clin Microbiol Rev 2019;33(1); doi: 10 .1128/cmr.00023-19
18. McIlwee BE, Weis SE, Hosler GA. Incidence of endemic human cutaneous leishmaniasis in the United States. JAMA Dermatol 2018;154(9):1032–1039; doi: 10.1001/jamadermatol.2018.2133
19. Makin S. A nasty tropical skin disease is now endemic in the U.S. n.d. Available from: https://www.scientificamerican.com/article/a-nasty-tropical-skin-disease-is-now-endemic-in-the-u-s/ [Last accessed: April 19, 2024].
20. World Health Organization. Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030: World Health Organization; 2020.
21. Taylor EM. NTD diagnostics for disease elimination: A review. Diagnostics (Basel) 2020;10(6):375; doi: 10.3390/diagnostics10060375
22. Bharadwaj M, Bengtson M, Golverdingen M, et al. Diagnosing point-of-care diagnostics for neglected tropical diseases. PLoS Negl Trop Dis 2021; 15(6):e0009405; doi: 10.1371/journal.pntd.0009405
23. Choi HL, Ducker C, Braniff S, et al. Landscape analysis of NTD diagnostics and considerations on the development of a strategy for regulatory pathways. PLoS Negl Trop Dis 2022;16(7):e0010597; doi: 10.1371/journal.pntd.0010597
24. Rogers MJ, McManus DP, Muhi S, et al. Membrane technology for rapid point-of-care diagnostics for parasitic neglected tropical diseases. Clin Microbiol Rev 2021;34(4):e00329-20; doi: 10.1128/CMR.00329-20
25. Avendaño C, Patarroyo MA. Loop-mediated isothermal amplification as point-of-care diagnosis for neglected parasitic infections. Int J Mol Sci 2020;21(21):7981; doi: 10.3390/ijms21217981
26. García-Bernalt Diego J, Fernández-Soto P, Muro A. LAMP in neglected tropical diseases: A focus on parasites. Diagnostics (Basel) 2021;11(3):521; doi: 10.3390/diagnostics11030521
27. Nateghi Rostami M. CRISPR/Cas9 gene drive technology to control transmission of vector-borne parasitic infections. Parasite Immunol 2020;42(9): e12762; doi: 10.1111/pim.12762
28. Wilson AL, Courtenay O, Kelly-Hope LA, et al. The importance of vector control for the control and elimination of vector-borne diseases. PLoS Negl Trop Dis 2020;14(1):e0007831; doi: 10.1371/journal.pntd.0007831
29. Raban R, Gendron WAC, Akbari OS. A perspective on the expansion of the genetic technologies to support the control of neglected vector-borne diseases and conservation. Front Trop Dis 2022;3; doi: 10.3389/fitd.2022.999273
30. Field MC, Horn D, Fairlamb AH, et al. Anti-trypanosomatid drug discovery: An ongoing challenge and a continuing need. Nat Rev Microbiol 2017; 15(4):217–231; doi: 10.1038/nrmicro.2016.193
31. Conceição-Silva F, Morgado FN. *Leishmania* Spp-host interaction: There is always an onset, but is there an end? Front Cell Infect Microbiol 2019;9: 330; doi: 10.3389/fcimb.2019.00330
32. Arango Duque G, Descoteaux A. Leishmania survival in the macrophage: Where the ends justify the means. Curr Opin Microbiol 2015;26:32–40; doi: 10.1016/j.mib.2015.04.007
33. Toepp AJ, Monteiro GRG, Coutinho JFV, et al. Comorbid infections induce progression of visceral leishmaniasis. Parasit Vectors 2019;12(1):54; doi: 10 .1186/s13071-019-3312-3
34. Távora LGF, Nogueira MB, Gomes ST. Visceral leishmaniasis/HIV co-infection in Northeast Brazil: Evaluation of outcome. Braz J Infect Dis 2015; 19(6):651–656; doi: 10.1016/j.bjid.2015.07.004
35. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, et al. Leishmaniasis: A review. F1000Res 2017;6:750; doi: 10.12688/f1000research.11120.1
36. Mann S, Frasca K, Scherrer S, et al. A review of leishmaniasis: Current knowledge and future directions. Curr Trop Med Rep 2021;8(2):121–132; doi: 10.1007/s40475-021-00232-7
37. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet 2018;392(10151): 951–970; doi: 10.1016/S0140-6736(18)31204-2
38. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: Clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis 2016;63(12):1539–1557; doi: 10.1093/cid/ciw742
39. Mishra J, Madhubala R, Singh S. Visceral and post-Kala-Azar dermal leishmaniasis isolates show significant difference in their *in vitro* drug susceptibility pattern. Parasitol Res 2013;112(3):1001–1009; doi: 10.1007/s00436-012-3222-1
40. Malecela MN, Ducker C. A road map for neglected tropical diseases 2021–2030. Trans R Soc Trop Med Hyg 2021;115(2):121–123; doi: 10.1093/trstmh/trab002
41. Perez-Molina JA, Molina I. Chagas disease. Lancet 2018;391(10115):82–94; doi: 10.1016/S0140-6736(17)31612-4
42. Lidani KCF, Andrade FA, Bavia L, et al. Chagas disease: From discovery to a worldwide health problem. Front Public Health 2019;7:166; doi: 10.3389/fpubh.2019.00166
43. Martín-Escolano J, Marín C, Rosales MJ, et al. An updated view of the *Trypanosoma cruzi* life cycle: Intervention points for an effective treatment. ACS Infect Dis 2022;8(6):1107–1115; doi: 10.1021/acsinfecdis.2c00123
44. Nunes MCP, Júnior MHG, Diamantino AC, et al. Cardiac manifestations of parasitic diseases. Heart 2017;103(9):651–658; doi: 10.1136/heartjnl-2016-309870
45. Saraiva RM, Mediano MFF, Mendes FS, et al. Chagas heart disease: An overview of diagnosis, manifestations, treatment, and care. World J Cardiol 2021;13(12):654–675; doi: 10.4330/wjc.v13.i12.654
46. Pérez-Molina JA, Crespillo-Andújar C, Bosch-Nicolau P, et al. Trypanocidal treatment of Chagas disease. Enfermedades Infecc Microbiol Clínica 2021; 39(9):458–470; doi: 10.1016/j.eimc.2020.04.011
47. Sales Junior PA, Molina I, Fonseca Murta SM, et al. Experimental and clinical treatment of chagas disease: A review. Am J Trop Med Hyg 2017;97(5): 1289–1303; doi: 10.4269/ajtmh.16-0761
48. Castro JA, de Mecca MM, Bartel LC. Toxic side effects of drugs used to treat Chagas' disease (American Trypanosomiasis). Hum Exp Toxicol 2006;25(8): 471–479; doi: 10.1191/0960327106het653oa
49. Rodriques Coura J, de Castro SL. A critical review on chagas disease chemotherapy. Mem Inst Oswaldo Cruz 2002;97(1):3–24; doi: 10.1590/s0074-02762002000100001
50. Cancado JR. Long term evaluation of etiological treatment of Chagas disease with benznidazole. Rev Inst Med Trop Sao Paulo 2002;44(1):29–37.
51. Crespillo-Andújar C, Comeche B, Hamer DH, et al. Use of benznidazole to treat chronic Chagas disease: An updated systematic review with a meta-analysis. PLoS Negl Trop Dis 2022;16(5):e0010386; doi: 10.1371/journal .pntd.0010386
52. Rijo-Ferreira F, Takahashi JS. Sleeping sickness: A tale of two clocks. Front Cell Infect Microbiol 2020;10:525097; doi: 10.3389/fcimb.2020.525097
53. Franco JR, Simarro PP, Diarra A, et al. Epidemiology of human African trypanosomiasis. Clin Epidemiol 2014;6:257–275; doi: 10.2147/CLEP.S39728
54. Anonymous. WHO interim guidelines for the treatment of gambiense human African trypanosomiasis. WHO Guidelines Approved by the Guidelines Review Committee. World Health Organization: Geneva; 2019.
55. De Koning HP. The drugs of sleeping sickness: Their mechanisms of action and resistance, and a brief history. Trop Med Infect Dis 2020;5(1):14; doi: 10.3390/tropicalmed5010014
56. Mesu VKBK, Kalonji WM, Bardonneau C, et al. Oral fexinidazole for late-stage African *Trypanosoma brucei* gambiense trypanosomiasis: A pivotal multicentre, randomised, non-inferiority trial. Lancet 2018;391(10116): 144–154; doi: 10.1016/S0140-6736(17)32758-7
57. Anonymous. Study Details. Efficacy and safety of fexinidazole in patients with Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei* rhodesiense. ClinicalTrials.Gov. n.d. Available from: https://clinicaltrials.gov/study/NCT03974178 [Last accessed: July 21, 2024].
58. Mesu VKBK, Kalonji WM, Bardonneau C, et al. Oral fexinidazole for stage 1 or early stage 2 African *Trypanosoma brucei* gambiense trypanosomiasis: A prospective, multicentre, open-label, cohort study. Lancet Glob Health 2021;9(7):e999–e1008; doi: 10.1016/S2214-109X(21)00208-4
59. Checchi F, Piola P, Ayikoru H, et al. Nifurtimox plus eflornithine for late-stage sleeping sickness in Uganda: A case series. PLoS Negl Trop Dis 2007; 1(2):e64; doi: 10.1371/journal.pntd.0000064
60. Kansiime F, Adibaku S, Wamboga C, et al. A multicentre, randomised, non-inferiority clinical trial comparing a nifurtimox-eflornithine combination to standard eflornithine monotherapy for late stage *Trypanosoma brucei* gambiense human African trypanosomiasis in Uganda. Parasit Vectors 2018;11(1):105; doi: 10.1186/s13071-018-2634-x
61. Wiedemar N, Hauser DA, Mäser P. 100 years of Suramin. Antimicrob Agents Chemother 2020;64(3); doi: 10.1128/aac.01168-19
62. Wiedemar N, Graf FE, Zwyer M, et al. Beyond immune escape: A variant surface glycoprotein causes suramin resistance in *Trypanosoma brucei*. Mol Microbiol 2018;107(1):57–67; doi: 10.1111/mmi.13854

63. Papagni R, Novara R, Minardi ML, et al. Human African trypanosomiasis (sleeping sickness): Current knowledge and future challenges. Front Trop Dis 2023;4; doi: 10.3389/fitd.2023.1087003
64. Kumeso VKB, Kalonji WM, Rembry S, et al. Efficacy and safety of acoziborole in patients with human African trypanosomiasis caused by *Trypanosoma brucei* gambiense: A multicentre, open-label, single-arm, phase 2/3 trial. Lancet Infect Dis 2023;23(4):463–470; doi: 10.1016/S1473-3099(22)00660-0
65. Loría-Cervera EN, Andrade-Narváez FJ. Animal models for the study of leishmaniasis immunology. Rev Inst Med Trop Sao Paulo 2014;56(1):1–11; doi: 10.1590/S0036-46652014000100001
66. Silva Pereira S, Trindade S, De Niz M, et al. Tissue tropism in parasitic diseases. Open Biol 2019;9(5):190036; doi: 10.1098/rsob.190036
67. Merida-de-Barros DA, Chaves SP, Belmiro CLR, et al. Leishmaniasis and glycosaminoglycans: A future therapeutic strategy? Parasit Vectors 2018; 11(1):536; doi: 10.1186/s13071-018-2953-y
68. McQuitty CE, Williams R, Chokshi S, et al. Immunomodulatory role of the extracellular matrix within the liver disease microenvironment. Front Immunol 2020;11:574276; doi: 10.3389/fimmu.2020.574276
69. Sutrave S, Richter MH. The truman show for protozoan parasites: A review of *in vitro* cultivation platforms. PLoS Negl Trop Dis 2021;15(8):e0009668; doi: 10.1371/journal.pntd.0009668
70. Benam KH, Dauth S, Hassell B, et al. Engineered *in vitro* disease models. Annu Rev Pathol 2015;10:195–262; doi: 10.1146/annurev-pathol-012414-040418 2015
71. Caddeo S, Boffito M, Sartori S. Tissue engineering approaches in the design of healthy and pathological *in vitro* tissue models. Front Bioeng Biotechnol 2017;5:40.
72. Porras AM, Hutson HN, Berger AJ, et al. Engineering approaches to study fibrosis in 3-D *in vitro* systems. Curr Opin Biotechnol 2016;40:24–30; doi: 10.1016/j.copbio.2016.02.006
73. He W, Reaume M, Hennenfent M, et al. Biomimetic hydrogels with spatial- and temporal-controlled chemical cues for tissue engineering. Biomater Sci 2020;8(12):3248–3269; doi: 10.1039/D0BM00263A
74. Tam RY, Smith LJ, Shoichet MS. Engineering cellular microenvironments with photo- and enzymatically responsive hydrogels: Toward biomimetic 3D cell culture models. Acc Chem Res 2017;50(4):703–713; doi: 10.1021/acs.accounts.6b00543
75. Grab DJ, Nikolskaia O, Kim YV, et al. African trypanosome interactions with an *in vitro* model of the human blood-brain barrier. J Parasitol 2004;90(5): 970–979; doi: 10.1645/GE-287R
76. Grifno GN, Farrell AM, Linville RM, et al. Tissue-engineered blood-brain barrier models via directed differentiation of human induced pluripotent stem cells. Sci Rep 2019;9(1):13957; doi: 10.1038/s41598-019-50193-1
77. da Silva Lara L, Andrade-Lima L, Magalhães Calvet C, et al. *Trypanosoma cruzi* infection of human induced pluripotent stem cell-derived cardiomyocytes: An *in vitro* model for drug screening for Chagas disease. Microbes Infect 2018;20(5):312–316; doi: 10.1016/j.micinf.2018.03.002
78. Almeida-Leite CMd, Galvão LMdC, Afonso LCC, et al. Interferon-γ induced nitric oxide mediates *in vitro* neuronal damage by *Trypanosoma cruzi*-infected macrophages. Neurobiol Dis 2007;25(1):170–178; doi: 10.1016/j.nbd.2006.09.003
79. Giobbe GG, Crowley C, Luni C, et al. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nat Commun 2019;10(1):5658; doi: 10.1038/s41467-019-13605-4
80. Jia Y, Wei Z, Zhang S, et al. Instructive hydrogels for primary tumor cell culture: Current status and outlook. Adv Healthc Mater 2022;11(12):e2102479; doi: 10.1002/adhm.202102479
81. Logullo J, Diniz-Lima I, Rocha JDB, et al. Increased *Trypanosoma cruzi* growth during infection of macrophages cultured on collagen I matrix. Life (Basel) 2023;13(4); doi: 10.3390/life13041063
82. Luz Y, Rebouças A, Bernardes CPOS, et al. Leishmania infection alters macrophage and dendritic cell migration in a three-dimensional environment. Front Cell Dev Biol 2023;11:1206049; doi: 10.3389/fcell.2023.1206049
83. Petropolis DB, Rodrigues JCF, Viana NB, et al. *Leishmania amazonensis* promastigotes in 3D Collagen I culture: An *in vitro* physiological environment for the study of extracellular matrix and host cell interactions. PeerJ 2014; 2:e317; doi: 10.7717/peerj.317
84. Fennema E, Rivron N, Rouwkema J, et al. Spheroid culture as a tool for creating 3D complex tissues. Trends Biotechnol 2013;31(2):108–115; doi: 10.1016/j.tibtech.2012.12.003
85. Zanoni M, Piccinini F, Arienti C, et al. 3D tumor spheroid models for *in vitro* therapeutic screening: A systematic approach to enhance the biological relevance of data obtained. Sci Rep 2016;6(1):19103; doi: 10.1038/srep19103
86. Yen BL, Hsieh C-C, Hsu P-J, et al. Three-dimensional spheroid culture of human mesenchymal stem cells: Offering therapeutic advantages and *in vitro* glimpses of the *in vivo* state. Stem Cells Transl Med 2023;12(5): 235–244; doi: 10.1093/stcltm/szad011
87. Scalise M, Marino F, Salerno L, et al. From spheroids to organoids: The next generation of model systems of human cardiac regeneration in a dish. Int J Mol Sci 2021;22(24):13180; doi: 10.3390/ijms222413180
88. Rodrigues AV, Alexandre-Pires G, Valério-Bolas A, et al. 3D-hepatocyte culture applied to parasitology: Immune activation of canine hepatic spheroids exposed to *Leishmania infantum*. Biomedicines 2020;8(12); doi: 10.3390/biomedicines8120628
89. Silberstein E, Kim KS, Acosta D, et al. Human placental trophoblasts are resistant to *Trypanosoma cruzi* infection in a 3D-culture model of the maternal-fetal interface. Front Microbiol 2021;12:626370; doi: 10.3389/fmicb.2021.626370
90. Silberstein E, Chung CC, Debrabant A. The transcriptome landscape of 3D-cultured placental trophoblasts reveals activation of TLR2 and TLR3/7 in response to low *Trypanosoma cruzi* parasite exposure. Front Microbiol 2023;14:1256385; doi: 10.3389/fmicb.2023.1256385
91. Rodríguez ME, Rizzi M, Caeiro LD, et al. Transmigration of *Trypanosoma cruzi* trypomastigotes through 3D cultures resembling a physiological environment. Cell Microbiol 2020;22(8):e13207; doi: 10.1111/cmi.13207
92. Rodríguez ME, Rizzi M, Caeiro L, et al. Transmigration of *Trypanosoma cruzi* trypomastigotes through 3D spheroids mimicking host tissues. Methods Mol Biol 2019;1955:165–177; doi: 10.1007/978-1-4939-9148-8_12
93. Garzoni LR, Adesse D, Soares MJ, et al. Fibrosis and hypertrophy induced by *Trypanosoma cruzi* in a three-dimensional cardiomyocyte-culture system. J Infect Dis 2008;197(6):906–915; doi: 10.1086/528373
94. Nisimura LM, Ferrão PM, Nogueira AdR, et al. Effect of posaconazole in an *in vitro* model of cardiac fibrosis induced by *Trypanosoma cruzi*. Mol Biochem Parasitol 2020;238:111283; doi: 10.1016/j.molbiopara.2020.111283
95. Ferrão PM, Nisimura LM, Moreira OC, et al. Inhibition of TGF-β pathway reverts extracellular matrix remodeling in *T. cruzi*-infected cardiac spheroids. Exp Cell Res 2018;362(2):260–267; doi: 10.1016/j.yexcr.2017.11.026
96. Torrico F, Gascón J, Barreira F, et al. New regimens of benznidazole monotherapy and in combination with fosravuconazole for treatment of Chagas disease (BENDITA): A phase 2, double-blind, randomised trial. Lancet Infect Dis 2021;21(8):1129–1140; doi: 10.1016/S1473-3099(20)30844-6
97. Torrico F, Gascon J, Ortiz L, et al. Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: A proof-of-concept, randomised, placebo-controlled trial. Lancet Infect Dis 2018;18(4):419–430; doi: 10.1016/S1473-3099(17)30538-8
98. Orlando LMR, Lechuga GC, da Silva Lara L, et al. Structural optimization and biological activity of pyrazole derivatives: Virtual computational analysis, recovery assay and 3D culture model as potential predictive tools of effectiveness against *Trypanosoma cruzi*. Molecules 2021;26(21):6742; doi: 10.3390/molecules26216742
99. Orlando LMR, Lara L da S, Lechuga GC, et al. Antitrypanosomal activity of 1,2,3-Triazole-based hybrids evaluated using *in vitro* preclinical translational models. Biology (Basel) 2023;12(9); doi: 10.3390/biology12091222
100. Harbuzariu A, Pitts S, Cespedes JC, et al. Modelling heme-mediated brain injury associated with cerebral malaria in human brain cortical organoids. Sci Rep 2019;9(1):19162; doi: 10.1038/s41598-019-55631-8
101. Sato T, Vries RG, Snippert HJ, et al. Single lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. Nature 2009;459(7244): 262–265; doi: 10.1038/nature07935
102. Lehmann R, Lee CM, Shugart EC, et al. Human organoids: A new dimension in cell biology. Mol Biol Cell 2019;30(10):1129–1137; doi: 10.1091/mbc.E19-03-0135
103. Chandrasegaran P, Nabilla Lestari A, Sinton MC, et al. Modelling host-*Trypanosoma brucei* gambiense interactions *in vitro* using human induced pluripotent stem cell-derived cortical brain organoids. F1000Res 2023;12:437; doi: 10.12688/f1000research.131507.2
104. Daghero H, Pagotto R, Quiroga C, et al. Murine colon organoids as a novel model to study *Trypanosoma cruzi* infection and interactions with the intestinal epithelium. Front Cell Infect Microbiol 2023;13:1082524; doi: 10.3389/fcimb.2023.1082524

105. Hughes D, Andersson DI. Evolutionary consequences of drug resistance: Shared principles across diverse targets and organisms. Nat Rev Genet 2015;16(8):459–471; doi: 10.1038/nrg3922
106. Kloker LD, Yurttas C, Lauer UM. Three-dimensional tumor cell cultures employed in virotherapy research. Oncolytic Virother 2018;7:79–93; doi: 10.2147/OV.S165479
107. Stoppini L, Buchs PA, Brun R, et al. Infection of organotypic slice cultures from rat central nervous tissue with *Trypanosoma brucei brucei*. Int J Med Microbiol 2000;290(1):105–113; doi: 10.1016/S1438-4221(00)80113-7
108. Postan M, Correa R, Ferrans VJ, et al. *In vitro* culture of cardiac mast cells from mice experimentally infected with *Trypanosoma cruzi*. Int Arch Allergy Immunol 2009;105(3):251–257; doi: 10.1159/000236765
109. Tanowitz HB, Brosnan C, Guastamacchio D, et al. Infection of organotypic cultures of spinal cord and dorsal root ganglia with *Trypanosoma cruzi*. Am J Trop Med Hyg 1982;31(6):1090–1097; doi: 10.4269/ajtmh.1982.31.1090
110. McCabe RE, Meagher S, Mullins B. *Trypanosoma cruzi:* Explant organ cultures from mice with chronic Chagas' disease. Exp Parasitol 1989;68(4):462–469; doi: 10.1016/0014-4894(89)90131-8
111. Hill KL. Biology and mechanism of trypanosome cell motility. Eukaryot Cell 2003;2(2):200–208; doi: 10.1128/ec.2.2.200-208.2003
112. Glogger M, Subota I, Pezzarossa A, et al. Facilitating trypanosome imaging. Exp Parasitol 2017;180:13–18; doi: 10.1016/j.exppara.2017.03.010
113. Dong L, Cornaglia M, Krishnamani G, et al. Reversible and long-term immobilization in a hydrogel-microbead matrix for high-resolution imaging of *Caenorhabditis elegans* and other small organisms. PLoS One 2018;13(3):e0193989; doi: 10.1371/journal.pone.0193989
114. Crilly NP, Mugnier MR. Thinking outside the blood: Perspectives on tissue-resident *Trypanosoma brucei*. PLoS Pathog 2021;17(9):e1009866; doi: 10.1371/journal.ppat.1009866
115. Oldenburg SH, Buisson L, Beneyton T, et al. Confining *Trypanosoma brucei* in emulsion droplets reveals population variabilities in division rates and improves *in vitro* cultivation. Sci Rep 2021;11(1):18192; doi: 10.1038/s41598-021-97356-7
116. De Niz M, Frachon E, Gobaa S, et al. Spatial confinement of *Trypanosoma brucei* in microfluidic traps provides a new tool to study free swimming parasites. PLoS One 2023;18(12):e0296257; doi: 10.1371/journal.pone.0296257
117. Uppaluri S, Heddergott N, Stellamanns E, et al. Flow loading induces oscillatory trajectories in a bloodstream parasite. Biophys J 2012;103(6):1162–1169; doi: 10.1016/j.bpj.2012.08.020
118. Stellamanns E, Uppaluri S, Hochstetter A, et al. Optical trapping reveals propulsion forces, power generation and motility efficiency of the unicellular parasites *Trypanosoma brucei brucei*. Sci Rep 2014;4:6515; doi: 10.1038/srep06515
119. Hochstetter A, Stellamanns E, Deshpande S, et al. Microfluidics-based single cell analysis reveals drug-dependent motility changes in trypanosomes. Lab Chip 2015;15(8):1961–1968; doi: 10.1039/c5lc00124b
120. Cadena MF, Rosero-Yanez G, Isa-Jara R, et al. Lab On A Chip (LOC) platform for drug screening against the intracellular forms of *Trypanosoma cruzi*. Microchem J 2024;203:110870; doi: 10.1016/j.microc.2024.110870
121. Vargas Jiménez A, Cabezas DCO, Delay M, et al. Acoustophoretic motion of *Leishmania* spp. parasites. Ultrasound Med Biol 2022;48(7):1202–1214; doi: 10.1016/j.ultrasmedbio.2022.02.016
122. Alrifaiy A, Lindahl OA, Ramser K. Polymer-based microfluidic devices for pharmacy, biology and tissue engineering. Polymers 2012;4(3):1349–1398; doi: 10.3390/polym4031349
123. Voyton CM, Choi J, Qiu Y, et al. A microfluidic-based microscopy platform for continuous interrogation of *Trypanosoma brucei* during environmental perturbation. Biochemistry 2019;58(7):875–882; doi: 10.1021/acs.biochem.8b01269
124. Sweileh WM. Contribution of researchers in Arab countries to scientific publications on neglected tropical diseases (1971–2020). Trop Dis Travel Med Vaccines 2022;8(1):14; doi: 10.1186/s40794-022-00173-7
125. Salje J, Weitzel T, Newton PN, et al. Rickettsial infections: A blind spot in our view of neglected tropical diseases. PLoS Negl Trop Dis 2021;15(5):e0009353; doi: 10.1371/journal.pntd.0009353
126. Fournier AMV, Boone ME, Stevens FR, et al. Refsplitr: Author name disambiguation, author georeferencing, and mapping of coauthorship networks with web of science data. R Package Version 2020;1(0.0).
127. Berger AJ, Linsmeier KM, Kreeger PK, et al. Decoupling the effects of stiffness and fiber density on cellular behaviors via an interpenetrating network of gelatin-methacrylate and collagen. Biomaterials 2017;141:125–135; doi: 10.1016/j.biomaterials.2017.06.039
128. DeForest CA, Kirkpatrick BE, Anseth KS. Engineering native biological complexity from the inside–out and outside–in. Nat Chem Eng 2024;1(1):2–5; doi: 10.1038/s44286-023-00013-1
129. Täuber S, von Lieres E, Grünberger A. Dynamic environmental control in microfluidic single-cell cultivations: From concepts to applications. Small 2020;16(16):e1906670; doi: 10.1002/smll.201906670
130. Ma C, Peng Y, Li H, et al. Organ-on-a-chip: A new paradigm for drug development. Trends Pharmacol Sci 2021;42(2):119–133; doi: 10.1016/j.tips.2020.11.009
131. O'Connor C, Brady E, Zheng Y, et al. Engineering the multiscale complexity of vascular networks. Nat Rev Mater 2022;7(9):702–716; doi: 10.1038/s41578-022-00447-8
132. Chung JJ, Im H, Kim SH, et al. Toward biomimetic scaffolds for tissue engineering: 3D printing techniques in regenerative medicine. Front Bioeng Biotechnol 2020;8:586406; doi: 10.3389/fbioe.2020.586406
133. Verrest L, Dorlo TPC. Lack of clinical pharmacokinetic studies to optimize the treatment of neglected tropical diseases: A systematic review. Clin Pharmacokinet 2017;56(6):583–606; doi: 10.1007/s40262-016-0467-3
134. Zushin P-JH, Mukherjee S, Wu JC. FDA modernization Act 2.0: Transitioning beyond animal models with human cells, organoids, and AI/ML-based approaches. J Clin Invest 2023;133(21); doi: 10.1172/JCI175824
135. McBlane JW, Phul P, Sharpe M. Preclinical development of cell-based products: A European regulatory science perspective. Pharm Res 2018;35(8):165; doi: 10.1007/s11095-018-2437-y
136. Ii YB, Burns BF, Siedner M, et al. Advancing equitable global health research partnerships in Africa. BMJ Glob Health 2018;3(4):e000868; doi: 10.1136/bmjgh-2018-000868
137. Larkan F, Uduma O, Lawal SA, et al. Developing a framework for successful research partnerships in global health. Global Health 2016;12(1):17; doi: 10.1186/s12992-016-0152-1
138. Adame F. Meaningful collaborations can end 'helicopter research.' Nature 2021; doi: 10.1038/d41586-021-01795-1
139. Chami GF, Bundy DAP. More medicines alone cannot ensure the treatment of neglected tropical diseases. Lancet Infect Dis 2019;19(9):e330–e336; doi: 10.1016/S1473-3099(19)30160-4

Received: May 09, 2024
Accepted: July 29, 2024
Online Publication Date: August 9, 2024