Building a community to engineer synthetic cells and organelles from the bottom-up

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On the challenges and opportunities in the field of synthetic cells and organelles – towards collaborative routes to overcome and reach them.

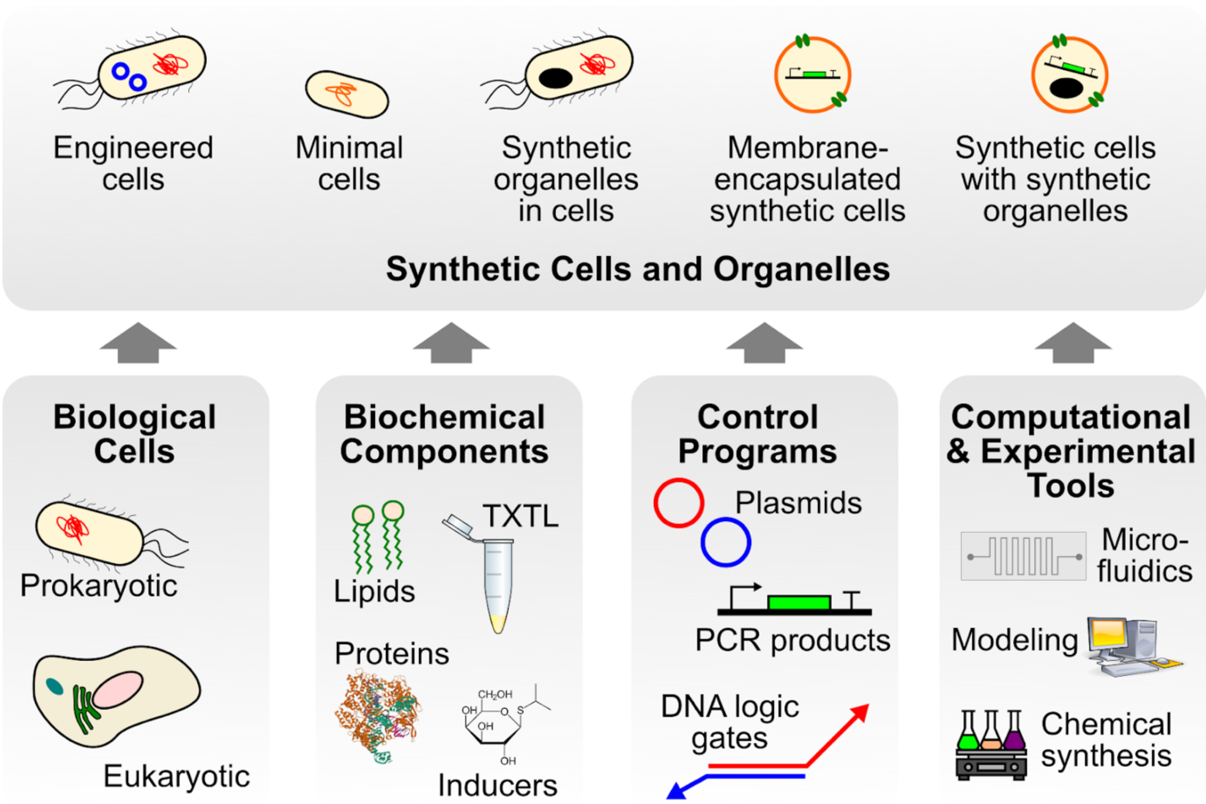
Abstract

Empowered by emerging concepts from physics, chemistry, and bioengineering, learning-by-building approaches have found increasing application in the life sciences. Particularly, they are directed to tackle the overarching goal of engineering cellular life from scratch. The SynCell2020/21 conference brought together a diverse group of researchers to share progress and chart the course of this field. Participants identified key steps to design, manipulate, and create cell-like entities, especially those with hierarchical organization and function. This article highlights achievements in the field, including areas where synthetic cells are having socioeconomic and technological impact. Guided by input from early-career researchers, we identify challenges and opportunities for basic science and technological applications of synthetic cells. A key conclusion is the need to build an integrated research community through enhanced communication, resource-sharing, and educational initiatives. Development of an international and interdisciplinary community will enable transformative outcomes and attract the brightest minds to contribute to the field.

Philosophy of the field and past achievements

Physicists and engineers traditionally focus on the non-living world and apply model systems with reduced complexity to capture the essentials of a system. Through this reduction in complexity, quantitative mechanistic insights into micro-, meso- and macroscale behavior of higher-order processes have been achieved. The past decade has witnessed the transfer of this approach into the life sciences, where a learning-by-building research strategy has resulted in the design and construction of synthetic cells and organelles of reduced but defined complexity[1]. As a result, construction of synthetic biological systems has received increasing attention, primarily based on discoveries to implement engineering principles in cell biological research[2]. Early national and transnational initiatives such as the Max Planck School "Matter to Life"[3], the Build-A-Cell research coordination network[4], the BaSyC research program[5], and the International Genetically Engineered Machine (iGEM) competition[6] have been established to advance research, training, and collaboration in this exciting new field.

Cells are the basic building blocks of life; however, their intricate structure and the tightly orchestrated interplay of individual molecular components within cells are far from basic. Most cellular phenomena are not understandable through intuition but require complex analytical systems to provide a mechanistic description of the processes forming living matter. Yet, within the complexity of living cells hide the answers to some of the most fundamental questions in the life sciences. Where is the transition from inanimate matter to life? How are biological structures organized across scales? How did life emerge on earth? How can man-made materials be integrated into living matter to direct behavior? How are diseases initiated, how do they develop, and how can they be mitigated? These compelling and profoundly difficult questions reflect a vision for the future of the field as expressed by the SynCell2020/21 early-career panelists. The philosophical and ethical considerations underlying these questions, e.g. the misuse of synthetic cells for biological warfare, the impact of synthetic cells on natural environments, or the unpredictable nature of completely new life forms, are notable for their contrast with technological and engineering-focused objectives[7]. Using principles from biology and engineering, interdisciplinary research teams have applied synthetic cells to construct materials and hierarchical structures with life-like properties that recreate essential features of living cells but also reach beyond the capabilities of natural cells (**Box 1**)[8-11].



Box 1 Research on engineering synthetic cells and organelles, as represented at SynCell2020/21, covers a wide range of experimental systems including engineered cells created using standard transformation techniques, minimal cells, membrane-encapsulated synthetic cells, and all of the above with the possible inclusion of engineered membraneless organelles that produce hierarchical structures. The common objective of the field is to engineer synthetic structures with defined complexity to mimic biological systems on multiple length scales. The creation and characterization of these experimental systems draws on a wide variety of interdisciplinary inputs, including biological cells, biochemical components (such as cell-free TXTL extracts), and control programs that encode desired behavior in a variety of formats. In addition, a broad range of computational and experimental tools are required.

Broadly speaking, there are two primary approaches to the construction of low-complexity synthetic cells: top-down and bottom-up[2]. Top-down approaches make use of existing living cells and sequentially remove individual components such as single genes[12]. This process can be iterated until reaching the absolute lowest point of complexity required for a cell to live – the bare minimum needed to run a living system. Analogous to synthetic lethality experiments in model organisms, top-down approaches provide descriptive insights into which parts of a cell are most crucial. However, it can be harder to obtain a systems-level understanding of how the parts work together. In contrast, the bottom-up approach rationally combines non-living molecules in an understand-by-design approach in order to activate and exhibit the behaviors of living cells within artificial structures[13]. A common defining element of cellular life forms is the ability to replicate a compartmentalized information-storing and self-sustaining out-of-equilibrium system that manifests itself in specific characteristics, which can be selected in an evolutionary process[14-16]. This could be manifested by engineering a compartmentalized entity, that exhibits a metabolism for reproduction purposes and environmental adaptation, e.g. a lipid membrane vesicle that undergoes growth and division by catabolizing exogenous substrates and harboring DNA encoded genes that specify the enzymes required for catabolism and reproduction. The advantage of the bottom-up approach is that every component of the created system can be located and defined in a quantitative manner, together with specified interactions between molecules. Regardless of the approach, top-down or bottom-up, the knowledge gained from building synthetic cells has the potential not only to provide fundamental insights into life, but also to result in technologies with global impact --- *e.g.*, new vaccination strategies[17], routes to overcome antibiotic resistance[18], new manufacturing pipelines for synthetic materials[19], and alternatives to petrochemicals[20].

Within the last decade, the field of building synthetic cells and organelles has achieved several major technological breakthroughs. By using a top-down approach, a minimal synthetic cell capable of metabolizing and reproducing has been constructed that contains only 473 genes.[21]. In this context, synthetic chromosomes have been designed to generate artificial genetic blueprints for operating synthetic cell systems[22]. Droplet-based synthetic cells with artificial photosynthetic metabolism have been produced and successfully applied to CO_2 fixation, and synthetic cell systems for the scalable bio-production of natural plant products have been demonstrated[23]. Bioengineering concepts emerging from the field have also provided novel means for the implementation of application-focused technologies. Most recently, synthetic genetic code expansions and technologies to rewire translational processes have provided the foundation for RNA-based SARS-CoV-2 vaccines[24,25]. Together with liposomal technologies, these vaccines have been a vital tool in the fight against COVID-19[26].

Exploring the fundamentals of life as illustrated in these brief examples requires diverse skill sets for designing and engineering experimental systems. It further requires an unbiased and creative mind concerning the perception of life given that aspects of physics, chemistry, biology, and the information sciences must be integrated as part of the research. Compared to other disciplines, engineering synthetic cells and organelles is exceptionally dependent on open-minded scientists possessing a strong interdisciplinary background. Students attracted to the field often share enthusiasm and interests that go beyond their primary disciplines, incorporating aspects of philosophy and cognition within their research (*e.g.*, the “Synthetic Biology, Politics, and Philosophy” workshop held at BrisSynBio[27]). This is in analogy to fields that explore artificial intelligence and neuromorphic computing[28]. This cross-fertilization across domains also provides a unique point of contact for attracting young researchers into the field of engineering synthetic cells and organelles (see **supplementary box 1** for selected quotes from young researchers attending the SynCell2021 Workshop).

Recent research directions and bottlenecks

The field of engineered synthetic cells and organelles has seen substantial progress within the last decade, especially in relation to the creation of compartmentalized cell-mimicking structures and the integration of coupled transcription-translation (TXTL) systems[29]. However, considerable challenges---some seemingly paradoxical---remain. Many of these were highlighted at the recent SynCell2020/21 conference (see Supplementary Information for listings of the conference program and links to recordings). One of the most demanding challenges is the coupling of information-encoding systems with self-replicating cell-like entities[30]. This challenge can be framed in terms of von Neumann´s abstract generality about the logic of cell-like self-replicating automata[31]. These not only require a mechanism to copy the cellular architecture itself but also functionalities that allow for the copying of (genetic) information specifying cellular structure and function. Implementation of such cell-like entities requires molecular systems engineering solutions that link the (i) functional parts of a synthetic cell to (ii) a decoding mechanism that reads the genetic instructions required to autocatalytically build a new cell and (iii) a molecular module that copies and reinserts a transcript of the (genetic) instruction into the synthetic daughter cell[32]. This is the logical basis of self-reproduction. Notably, the first steps towards the man-made construction of such systems were presented at the conference. DNA-encoded genetic systems represent just one particular implementation of self-reproduction, leaving ample room for designing alternative means of fulfilling the basic conditions for a “living” synthetic cell[33,34]. In addition, synthetic cells will also require control programs to orchestrate the interconnected processes of sensing, response, and metabolism required for self-replication and the other processes needed to give life-like behavior to synthetic cell systems[35-37].

Important progress reported at the meeting also included the engineering of synthetic structures with hierarchical organization inspired by eukaryotic life forms. Several implementations of such systems, *e.g.* hierarchical intrinsically disordered protein droplets generated within synthetic cell-like compartments, were presented[38]. These efforts are aimed at deconvolving the organizational principles of life, including the highly dynamic cross-scale architecture of eukaryotic and multicellular organisms, most apparent during embryogenic development and tissue regeneration. Such questions have puzzled biologists for decades. How the structural organization of subcellular, cellular and tissue components is hard-wired and how degrees of plasticity in respective structures are regulated, are problems of such immense complexity for which, to date, approaches including multi-centered global screening efforts have not been able to resolve the underlying principles. Novel methods based on lower-complexity *in vitro* reconstituted synthetic model systems may provide new insights into these processes. For example, a pivotal driving force behind tissue organization consists of genetic feedback loops based on reaction-diffusion processes and hysteresis, as first proposed by Alan Turing in his work on the chemical basis of morphogenesis in the mid-20th century[39]. This is a prime example of how reductionist approaches in the form of precisely-defined models can be applied to the study of complex behaviors in biological systems. Researchers in synthetic biology have recently recreated Turing patterns from protein-based systems and used these to study decision-making during cellular organization and symmetry breaking[40]. This not only underscores the fundamental impact of the questions asked in the field but also their longstanding relevance that argues for the need to pursue novel theoretical, computational and experimental approaches by unbiased young scientists working in integrative research communities.

Other approaches have contributed insights into the spatio-temporal dynamics and organization principles of membraneless organelles[38]. Until recently, studying such dynamic structures in living cells has mostly been limited by a lack of perturbation capabilities and the undefined chemical environment within the cytosol. However, through *in vitro* reconstitution of intrinsically disordered protein systems in isolated low-complexity environments, quantitative insights into the molecular and thermodynamic principles needed for assembly and homeostasis of phase-separated organelles has been achieved[41]. Understanding the hierarchical organization principles of life will ultimately enable the formulation of the principal laws of decision-making within living matter, and the basis of information processing and signal integration within cell collectives[42,43].

Engineering synthetic cells and organelles is not solely directed towards investigating biological principles, but also holds promise for practical applications. For engaged young researchers, this offers the opportunity to explore an extensive technical repertoire. For instance, microfluidic approaches have been developed for the assembly of synthetic cells with adjustable and tunable composition. Specifically, water-in-oil droplets as cell-iszed compartments are generated and their lumen is filled with proteins, lipids or nucleic acids, providing means to engineer systems capable of genetic information processing and artificial genotype-to-phenotype coupling[23,44-47]. Droplet-based microfluidic approaches have also been adopted for lipid membrane engineering[37,48]. Similarly, DNA nanotechnology has allowed to combine programmable molecular architectures with extrinsically controlled functions[49,50]. In a combinatorial approach, integration of DNA nano-architectures with synthetic cells has synergized top-down and bottom-up strategies[51]. These examples demonstrate the potential for technology innovation originating from the field.

Future perspectives and community

To the extent that living cells are modular, engineering approaches set the stage for implementing synthetic functional modules capable of performing specific functions in synthetic cells. The successful combination of all individual elements within a single entity will be key for the assembly of synthetic living cells. This, in turn, requires integrated inter-laboratory solutions that allow for off-the-shelf unification of individual modules. Exchanging expertise between laboratories and universal module interfaces will be essential.

Discussions during SynCell2020/21 revealed several fundamental strategic frameworks and infrastructure that will need to be implemented in order to achieve the successful integration of the global community:

(I) In the interest of effective paywall-free knowledge transfer among researchers, open-access data repositories are needed. This will not only facilitate transfer of experimental protocols but also sharing of data and blueprints for synthetic cell modules, effectively boosting access of interested students to the field. Moreover, standardization efforts that strive to provide universal norms for the design and assembly of synthetic cell modules and interfaces in a plug-and-play manner need to be developed. Specific implementations of such platforms could be arranged, inspired by the collaborative software development and version control platform GitHub that has experienced community-wide acceptance within computer science and engineering fields. The Build-A-Cell network has embraced this approach, and has begun to assemble such open-access repositories[4].

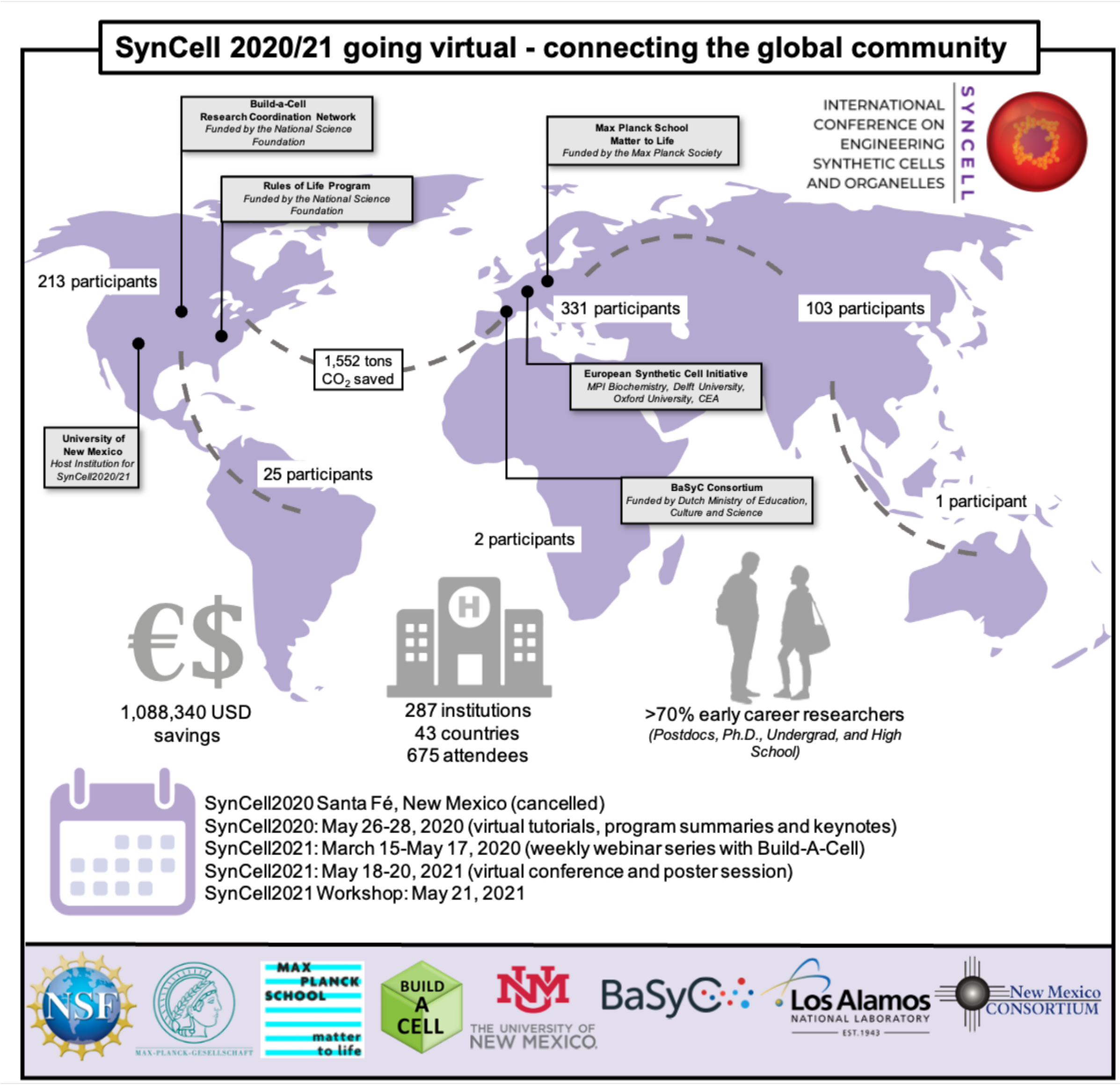
(II) Engineering synthetic cells and organelles will be a model for new transcontinental educational modalities. SynCell2020/21 was jointly organized between the National Science Foundation (USA) and the Max Planck Society (Germany). Moreover, it received support from national research programs, e.g. the Build-A-Cell network (USA based) and the BaSyC program (Netherlands) (**Online-only box 2**). A focus of the conference framework were presentations by leading researchers in student-centered tutorials. Community-driven education programs for specialized training in relevant domains (biology, physics, chemistry, microbiology, molecular biology, biophysics, computer science, ethics) will be key for equipping new generations with the necessary skills for succeeding in engineering living synthetic cells and organelles. International workshops and research summer schools will be important in the development of a coherent, long-lasting community that fosters cross-generational collaborations among scholars. At present, only a limited number of training and graduate programs focused on the engineering of synthetic cells and organelles have been established, such as the Max Planck School Matter to Life, the Cold Spring Harbor Laboratory summer school on synthetic biology and research programs supported by the National Science Foundation Rules of Life initiative. Their successful implementation will not only nurture the next generation of scientists but will also train a cohort of researchers to enable industrial applications. If possible, future events should be organized between all major research and teaching initiatives (see online-only box 2) in order to bring together the global expertise.

(III) Following the understanding-by-designing approach, the field awaits a steadily growing demand for an integrated research infrastructure that provides computational power and specialized courses in molecular and genetic design. This includes molecular modelling of large-scale whole-cell models for predicting the interactions of engineered components with host cells. Access to advanced computational facilities as well as enhanced algorithms for simulations based on machine learning and optimization techniques will greatly expand the scope for design and construction of synthetic cells and organelles. Dedicated research centers, such as the Max-Planck-Bristol Center for Minimal Biology[52], could provide such facilities as well as technical support concerning increasingly important administrative aspects to the field, *e.g.* technology transfer procedures, handling of intellectual property issues, and curation of specialized genetic parts and molecular module libraries specified for the field (inspired by biobanks such as Addgene and large-scale gene and genome synthesis "biofoundries" such as those funded by the Department of Energy in the US).

For all proposed the measures, commitment and support from funding bodies, political and regulatory authorities as well as universities with established teaching infrastructure will be essential. This is most important for the successful installation of a strategic open-source platform for synthetic biology and student exchange programs as now established between the University of New Mexico and the Max Planck Society. Moreover, private and philanthropic foundations could provide financial support for these actions (e.g., the "Life?" program by the Volkswagen Foundation).

Specific measures will include a joined program between the research initiatives mentioned in II, aimed towards continued organization of the SynCell conference as a think-tank for community building and research exchange. Moreover, the Build-a-Cell initiative has initiated several focused working groups, e.g. working towards collection and annotation of synthetic cell subtype components or towards establishing *in silico* modeling frameworks of synthetic cells with predictable behavior[53]. These groups provide an optimal platform to develop future cross-scale-organized infrastructure that will be able to manage between different stakeholders from academia, industry and political authorities, while also serving as an advisory council representing the field's interest. Furthermore, concentrated efforts will be made to raise awareness in academic faculties and scientific societies towards the importance of establishing relevant teaching schemes in graduate and undergraduate programs.

A compelling model for developing and sharing modular tools across the diverse synthetic biology community can be found in the design of the original Unix multi-user operating system (OS)[54] and subsequent community-driven, evolutionary development of Linux[55]. Unix's "graceful facilities" enabled users to create complex programs from existing modules through the novel use of pipelining[56]. At the same time, the OS was designed to facilitate communication among programmers as "the essence of communal computing"[56].Linux emerged from an unprecedented, worldwide open-source effort by volunteer programmers. These core values of streamlined, modular design and enthusiastic, open, collaborative development can similarly inform and shape progress in the synthetic cell community.



Online-only box 2 The International Conference on Engineering Synthetic Cells and Organelles was originally scheduled to take place in Santa Fé, New Mexico (USA) in 2020 with 150 participants. The global pandemic necessitated the presentation of the program over the next year in a free, virtual format (SynCell2020/21), which greatly enhanced global participation. The world map depicts the origin and diversity of the participants as well as the major research and educational initiatives in the field of engineering synthetic cells and organelles. Logos indicate organizations that made the conferences possible.

Conclusion

SynCell2020/21 demonstrated the remarkable engagement of a large and geographically-diverse community as well as the potential for global collaboration and transcontinental knowledge-sharing as the foundation for future success in the field. Importantly, a collaborative and well-trained community, including a new generation of young scholars, will be able to responsibly and effectively communicate the societal impacts of engineering synthetic cells and organelles to the public, particularly with respect to questions of how to share intellectual property to benefit humanity while continuing to reward innovation, biosafety, biosecurity and other unique ethical and philosophical considerations, including the most fundamental question of all: “what is life”?

Supplementary Information

Supplementary tables provide lists of presentations comprising SynCell2020/21 activities, along with links to recordings posted on YouTube.

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O.S., S.R.A., M.R.L., A.P.S. and G.P.L. conceptualized the manuscript and figures.

O.S. wrote, and S.R.A., M.R.L., A.P.S. and G.P.L edited the initial drafts of this manuscript.

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