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VIEW

MINI-REVIEW

New bioimaging avenues for organs-on-chips by integration of bioluminescence

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Abstract

The unmet demand for the development of advanced in vitro models that emulate key features of human (patho-)physiology and organ or tissue functionality led to the emergence of organ-on-a-chip screening platforms. This biomimetic microtechnology enables accurate prediction of human responses, even at the multitissue or organ interaction level. With these advances, the necessity of incorporating biosensing within these platform becomes increasingly evident, to ultimately allow in situ, noninvasive, monitoring of cells, tissues and organs' behavior. Seamlessly integrated biosensors on-chip also offer the prospect of uninterrupted and fully automated analysis. The continuous search for biosensors that not only avoid disruptive analyses, but are also highly sensitive and permit instant in situ evaluation, steered further developments of optical biosensors including bioluminescence (BL). BL is particularly suitable to interrogate biological systems at the multiparameter level and it is well matched to address the intrinsic biological systems' heterogeneity due to its inherent high signal to background ratio, to which the demand of simple measurement equipment can be added. Moreover, BL can be effortlessly integrated into any available organ-on-a-chip platform and provides unique groundbreaking means for online monitoring, at the cellular level, and/or detection of cell secreted/excreted compounds. This review depicts recent advances in biosensors, with particular focus on BL. We highlight current challenges and future directions, aimed at stimulating the application of BL to interface organ-on-a-chip systems, leading to the exponential advancement of both fields.

KEYWORDS
bioluminescence, drug screening, microfluidics, microscopy, optical imaging, organ-on-a-chip

1 | INTRODUCTION

Organ-on-a-chip technology (also named as microphysiological systems) has recently emerged as a new alternative to nonrepresentative animal models and oversimplified cell culture models. These advanced in vitro models of human (patho-)physiology provide a unique and complexity-balanced approach, while enabling a strict

control over tissue/organs microarchitecture and key functions, carefully selected to closely emulate corresponding human native tissues and organs.[1] These microphysiological systems seamlessly integrate microfluidics, microfabrication, biology, tissue engineering, materials and (bio)imaging/sensing.[2] Ultimately, these 3D models offer the potential to meaningfully and significantly expand our understanding of human diseases, while providing drug development and therapy screening a faster and more successful pace. Altogether, these features render this approach cost-effective and patient-specific.[3–6]

One of the most promising tracks toward generalized acceptance and widespread application of organ-on-chip devices by technology developers (eg, academic, clinical, industrial) and stakeholders (eg, pharmaceutical companies, regulatory entities, cosmetic and food industry), comprises the integration of comprehensive and mechanistic analysis, ideally in a noninvasive, real-time, straightforward and automated fashion.[7] Diverse integration strategies of detection, either via optical or nonoptical sensors, have been reported during the last decade.[8] Among them, optical biosensors based on chemical luminescence detection, namely bioluminescence (BL), are undoubtedly highly promising ones, and, thus, reviewed in this article. BL is exceptionally attractive due its intrinsic high signal-to-noise ratio, equipment simplicity, and multiplexing prospects.[9] BL-based assay can reach limit of detections of attomol (as in case of ATP assay),[10] or of nanomols for detection of molecules with relevant biological function[11] and linear range of for 6 to 8 logs of analyte concentration. Despite the myriad of achievements reporting on in vitro and in vitro application of BL-based detection and analysis, it seems that the integration of these approaches in microfluidic devices remains negligible.[12–17] The main challenges linger on the integration and optimization of analysis functions into noninvasive fully autonomous microsystems, in a cost-efficient and robust manner. The main aim of this review is to highlight the potential of integration of BL strategies with organ-on-a-chip systems, emphasizing on possible applications, design requirements, detection ranges, and further foreseen advancements.

2 | MONITORING ORGAN-ON-A-CHIP SYSTEMS USING BIOSENSORS

Standard analytical methods typically demand large volumes of samples, liquids, or cells, normally correlated with significant disturbance within the cell culture platforms (including end-point analysis), rendering these conventional methodologies rather incompatible with organ-on-a-chip systems.[18] Embedded detection systems, such as biosensors, are, therefore, essential for continuous in situ monitoring of the status of the microphysiological systems, over short or long time frames and in an automated manner.[19] A large number of well-established biosensors (ranging from micro- to nano-scale) can be effortlessly fitted specifically for organ-on-a-chip models, which enable overcoming limitations associated with end-point assays (eg, limited kinetic and/or prognostic information).[20] Nevertheless, so far, only a very narrow number of studies have focused on the unique combination of analytical biosensor methods and organs-on-chips. The integrated analytical techniques so far implemented in organs-on-chips are primarily based on (electro-)chemical, optical, and optochemical sensors to characterize cell behavior, intra- and intertissue/organ communication or interfaces (as depicted in Figure 1A and B). For example, transendothelial or transepithelial electrical resistance (TEER) and electric cell-substrate impedance sensing measurements are mainly used to assess the integrity of human endothelial or epithelial barrier models and cell's activity based on cytoskeleton changes (eg, morphology and locomotion), respectively.[21–24] Other methodology commonly integrated in organ-on-a-chip systems consists of multielectrode arrays, which allow for electrophysiological interrogation of cell activity, ideal for studying neural networks.[25,23] Additional electrochemical and optochemical sensors include oxygen detection, pH levels, ions and consumption/release of metabolic compounds, such as glucose, ammonia, and lactate,[27,28] as also depicted with the relevant example in Figure 1C. Notwithstanding these options, the vast majority of organ-on-a-chip current readouts are microscopy-based, typically combined with sacrificial off-chip analytical techniques (eg, qPCR, FACS, mass spectroscopy, and ELISA).[28] Optical methods offer several advantages, which render them highly suitable for merging with organ-on-a-chip platforms, such as being nondestructive, robust, sensitive, and, importantly, compatible with in situ and/or inline monitoring,[29] as elegantly depicted in the example in Figure 1D. Well known by the required mild conditions and noninvasiveness, luminescence-based biosensors are based on imaging light emission resulting from thermo, electrogenerated-, or chemi-BL reactions. These and other relevant examples have been exhaustively described in recent literature, thereby not the focus of the current overview.[30–33]

2.1 | Nonmicroscopy-based biosensors

Organ-on-a-chip incorporated nonmicroscopy dependent biosensors are mostly electrochemical based. The development of integrated sensors on-chip allows for inline evaluation of several key aspects of cells, tissues, or organs'

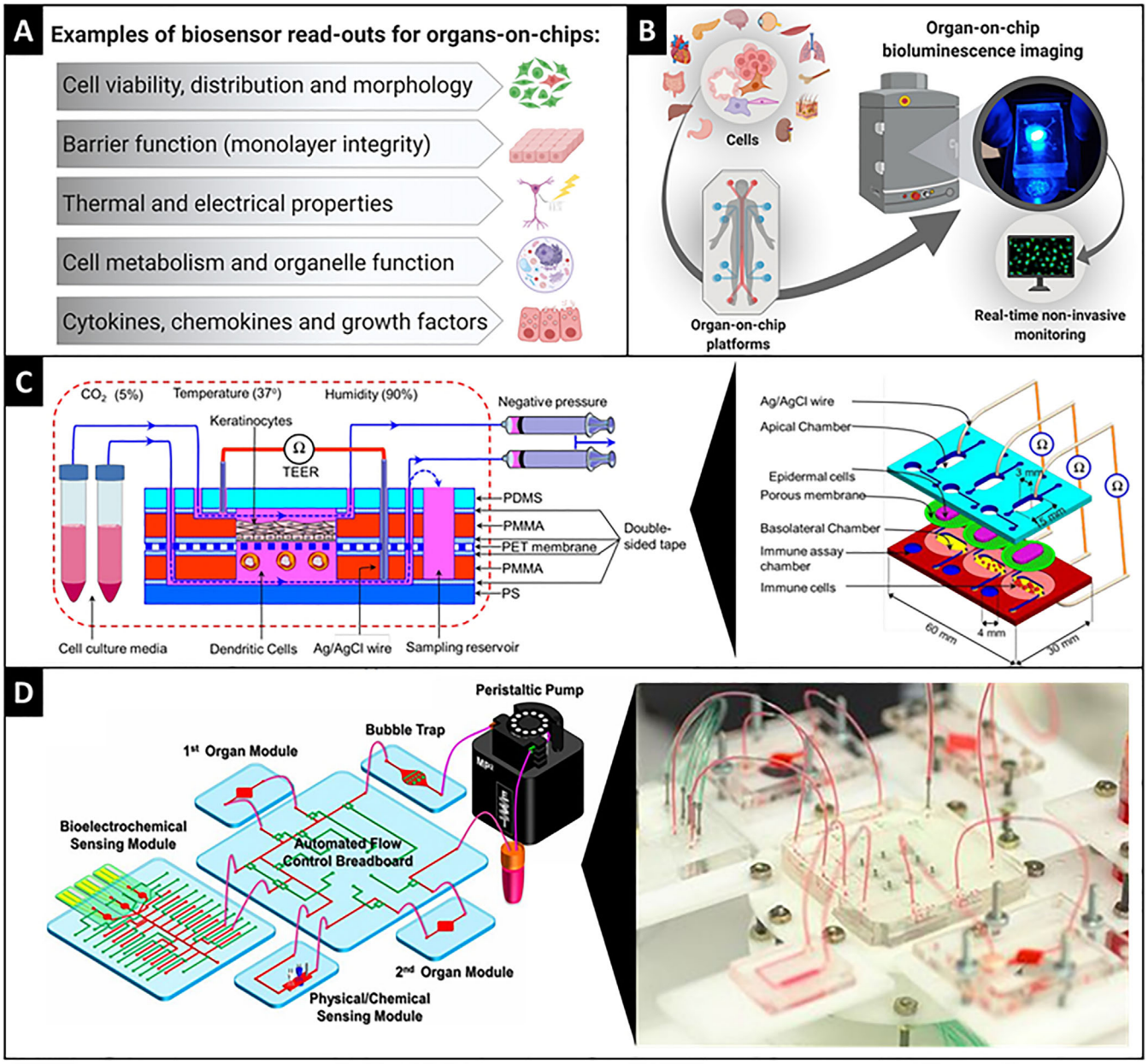

FIGURE 1 Biosensors and organs-on-chips. **(A)** Examples of typical biosensor readouts integrated in organs-on-chips platforms. **(B)** Schematic diagram showing the seamless embedding of organs-on-chip platforms with bioluminescence imaging. As depicted, organ-on-a-chip technology is based on three key pillars: cells, microfluidic technology, and biomarker detection/analysis. Created with BioRender.com. **(C)** Representative example with inset of an integrated modular microfluidic device composed by: microbioreactors, breadboard, reservoir, bubble trap, physical sensors, and electrochemical biosensors. Adapted from;[31] Copyright (2017) National Academy of Sciences. **(D)** Depiction of a schematic cross-sectional view (left side) and overview (right side) of a 3D organ-on-a-chip device with three parallel cell culture chambers and incorporated TEER measuring electrodes. Adapted with from,[100] with permission by Royal Society of Chemistry.

functionality, including (a) assessment of formation/disruption of barrier function; (b) detection of cell communication signals or secreted signaling molecules; and (c) monitoring of complex biotransformation processes or absorption, distribution, metabolism, excretion, and toxicity screens. Although the full integration of electrodes within organ-on-a-chip platforms is still not a very straightforward process, several publications report on successful cases of embedded electrodes to typically monitor, in real time and in a noninvasive manner, for example, the barrier function of cultured cells or tissues. As representative example, integrated, semitransparent, sensing electrodes to measure TEER have been recently reported, by using simple layer-by-layer fabrication

process, to measure human epithelial barrier function.[34] In this study, integrated electrodes enabled the assessment of formation/disruption of barrier function in a lung airway-on-chip and in a gut-on-chip model, lined by intestinal epithelial cells. Another relevant study emphasized on the development of microfluidic cocultures with integrated biosensors, intended to monitor secreted signals locally and in a continuous mode.[35] This study was based on a liver injury-on-a-chip model, where a microfluidic coculture system has been integrated with biosensors for monitoring liver cell signaling to assess effects of injury. With these integrated miniaturized biosensors, it was possible to monitor paracrine cellular crosstalk by a common signaling molecule. Similarly, it has been recently reported that embedded electrochemical sensors are efficient alternatives for conventional end-point assays, via glass chip-based embedded TEER and reactive oxygen species sensors, to address fibrosis on-chip.[36] Other studies reported on sensorized on-chip systems with integrated electrothermal micropumps and sensors to study cell behavior, namely monitoring cell adhesion and proliferation, oxygen consumption rates of cells, and pH detection to prevent adverse physiological effects by the cellular acidification.[37] This level of sensor complementarity was enabled by combining platinum structures to monitor cell proliferation, amperometric sensors to detect oxygen consumption, and potentiometric pH sensors (via Si3N4 layers). The integration of metabolic profiling biosensor mediated with microtechnology, contributes to the development of "metabolomics-on-a-chip" platforms, with important applications in both pharmaceutical and environmental toxicology. As demonstration of this metabolic profiling on-chip, mitochondrial respiration was monitored in real time using two-frequency phase modulation, providing real-time contiguous electrochemical measurements of glucose and lactate.[26] With such measurements, it was possible to detect shifts from oxidative phosphorylation to anaerobic glycolysis, thereby enabling early detection of mitochondrial stress. This was also achieved by Zhang et al., elegantly showing that by integrating a wide variety of real-time sensors on-chip, it was possible to achieve fully automated in situ monitoring of both biophysical and biochemical parameters.[19] Altogether, multiple nonmicroscopy-based biosensors have proven to be successful and easily integrated with micro- or nano-systems. However, only more recently, studies start demonstrating that analytical approaches can be effectively combined with organs-on-chips. This may be due to the intrinsic limitations of these type of biosensors, namely restricted 3D information on specific location/cellular event due to averaged overall measurements rendering these techniques mostly unsuitable to evaluate events at the cellular level. Moreover, most of current nonmicroscopy-based biosensors may not be available or even compatible with online setups, may be too demanding regarding sample preparation and/or may fall short on specificity, when high protein content leads to high background noise.[38] To circumvent this limitation, BL measurement, for example, via sampling output media, can be easily integrated in any type of chip. In fact, many luciferases can be expressed in cells as secreted form or the conversion of a proluciferin substrate into luciferin, in response to a metabolite that can be measured in a luminometer by simple addition of purified luciferase and other cofactors.[39,40]

2.2 | Microscopy-based biosensors

Integrating optics with microfluidics is not a new concept. Actually, it has been established as a new field, named optofluidics. The optofluidics methods mostly applied as biosensors for microfluidics measurements can be divided into two general categories: label-free and label-based methods. Label-free methods include surface plasmon resonance,[41,42] scanning electron microscopy,[43] and Raman microscopy.[44] Although these methods allow the study of specific cell's secreted/excreted compounds, cellular morphology or targeting of specific areas of individual cells for analysis, respectively, some shortcomings are intrinsically associated, namely possible interference with conformation of molecular structures, interaction with biological processes, and demand for bulky external equipment. Additionally, when the microfluidic chips are polydimethyl siloxane-based, some additional challenges may be presented such as retrieving and/or processing the samples for analysis. On the other hand, the most common labelling-based methods applied on-chip are fluorescence-based or chemical luminescence-based biosensors. It is widely accepted that from all optical methods, fluorescence sensing is still the most popular detection method used in organ-on-chip platforms.[29] Indeed, most of the readouts compatible with organs-on-chips so far reported in literature remain heavily dependent on fluorescence-based microscopy assessment.[45–47] Fluorescence-based analysis is typically reliant on prelabeled cells or demand invasive end-point procedures, such as cell fixation, necessary for further processing for immunohistochemistry or histological staining.[48] Successful strategies to minimize process cumbersomeness, variability and user bias have been reported using an automated workflow, enabling acquisition and analysis of confocal images of organs-on-chips within shorter time frames.[49] In this study, thorough cellular phenotype profiles were determined in large sample numbers, permitting improved statistical power, highly relevant for decision making for

routine testing in drug screening dependent on quantitative image data. Others reported on live imaging platforms of whole microorganism models, namely worms, allowing multiplexed longitudinal surveillance of various biological processes.[50] This approach can undoubtedly be translated into organ-on-a-chip platforms. Combined optical imaging has also been reported using a bone-on-chip system to study osteogenic differentiation in vitro.[51] In this interesting coupled approach, the monitoring of cell viability, proliferation, and differentiation was determined using both fluorescence and BL, allowing nonterminal imaging. Noteworthy, although widely used, fluorescence-based detection may face limitations associated with cells or tissue damage due to photobleaching. Possible disparities of photobleaching rates at several locations within the cells may also compromise long-term monitoring. As an alternative to circumvent this limitation, BL resonance energy transfer (BRET) has been employed on microfluidic devices for measurement of thrombin activity.[52] This approach consists of a milder form of bioanalytical sensing, that supports lower limits of detection, when compared to fluorescence resonance energy transfer.

3 | TOWARD NONINVASIVE MOLECULAR OPTICAL ANALYSIS

3.1 | State-of-the-art bioluminescence imaging

BL substantially differs form fluorescence where the intensity of the emission is dependent on the average excitation power. Although the fluorescence emission can be relatively higher than BL, it suffers from significant net background due to presence of weak fluorophores present in biological samples. For BL to occur, excitation light is not required, thereby background emission is mostly negligible, which leads to high signal/background ratio.[53] The high detectability of BL resulted in increased popularity for use in bioanalytic assays, since sensitivity can be improved over 10 to 10 000 times, when compared to fluorescence.[53] Moreover, BL observation lacks the phototoxicity problems and permits the long-term, nonlethal observation of living cells, from 2D cell layers to more complex 3D assemblies including organoids, embryos, and worms.[54] On the other hand, BL reaction requires a substrate that should be preferentially stable and ensure enough diffusion through multiple cell membranes, as in the case of layers of cells typically composing organ-on-a-chip models.

In the last decade, scientists developed novel and relevant bioluminescent systems based on mutagenesis of natural enzymes. These can either be derived by different luminous organisms or generated synthetic luciferin substrates.[55] Also importantly, multicolor emission and increased brightness have been achieved, which permits visualization of multiple cell types cocultured on-chip.[56] For comprehensive enumeration of luciferases system available for eukaryotic expression, the readers can refer to multiple recent reviews in the field,[9,55–57] while here we focus on the BL imaging systems that are better suited for imaging on-chip due to their brightness.

Luciferase genes from terrestrial organisms have been cloned and characterized mostly from insects such as fireflies, click-beetles, and railroad worms. Jamaican click-beetle-derived luciferases (CBG99, CBG2) and Brazilian click-beetle-derived luciferase (emerald luciferase Eluc from *Pynearius termilluminans*) and a red-emitting luciferase derived from Railroad worm (stable luciferase red, SLR) are bright enzymes for imaging single cells using microscopy and D-luciferin,[58] and outperform standard firefly luciferase. However, pairing firefly luciferase with modified luciferins, namely cyclic luciferins (CycLuc) and Cybluc, charactherized by high cell membrane permeability, would undoubted improve the perfomance of Fluc in a wide range of bioimaging applications.[59,60] A recent development is the AKABLI system based on synthetic Akaluc luciferase and luciferin analogue Akalumine.[61] Due to the 28 amino acids difference from Fluc, Akaluc demonstrated to be brighter than than Fluc/D-luciferin (λmax 650 nm).[62] Optimized marine luciferases also yield bright BL. For example, *Gaussia princeps* luciferase (GLuc), *Metridia longa* luciferase paired with coelenteraziene[63,64] and Nanoluc luciferase with its furimazine substrate,[65] Teluc with its diphenylterazine (DTZ) substrate[66] demonstrated 100- to 1000-fold higher emission than firefly/D-luciferin in live cells. Other marine luciferase inspired enzymes based on intramolecular BRET between the bioluminescent donor and a fluorescent acceptor protein have also been generated.[67,68] A plethora of novel coelenterazine analogues have also been synthetized mostly focusing on the modification of the C-2, C-5, C-6, and C-8 substituents of the imidazopyrazinone core, with the most effort on modifying the C-2 and C-6 position.[69,70] Some of the analogues reacting with Nanoluc showed improved bioluminescent properties in terms of signal intensity and duration of the reaction. Hikarazines-003 produced with up to 2.5 increased light intensity and signal stability lasting up to 2 h.[71] The recently developed 5-fluorofurimazine also increased light output of threefold, in living cells cultured in vitro.[72,73] Caging by addition of protecting groups, as the C-3 carbon of the imidazopyrazinone core, is a common strategy to stabilize coelenterazine-derived substrates. Other two analogues, based on caging strategy, resulted in significantly longer BL emission with higher *S/N* ratio than furimazine at single-cell level, namely

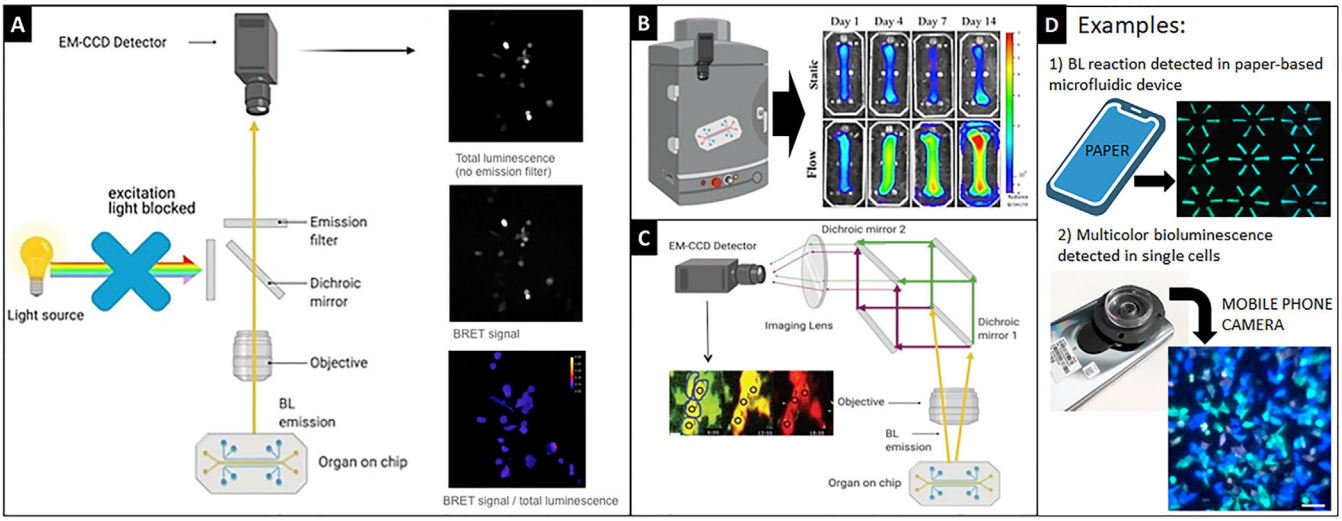

FIGURE 2 Bioluminescence imaging of organs-on-chips. **(A)** Typical set-up of a microscope for detection of bioluminescence (left panel). A sensitive EM-CCD camera is employed and light source is blocked. Detection of bioluminescence from single cells, cultured in a chamber slide, expressing Antares (a fusion between Nanoluc and CYoFP orange fluorescence protein) and detection of BRET signal using a red filter (right panels). **(B)** Detection of bioluminescence from an organ-on-a-chip using an imager equipped with cooled CCD camera. Luminescence from cells expressing firefly luciferase cultured in the chip is used as an indication of viability (right panel adapted from Sheyn et al[101]) in response to drug treatment. **(C)** Set-up of a BL microscope for detection of multicolor luciferases using dichroic mirrors. Dual gene expression dynamics in single cells has been achieved from cell culture in a dish (adapted from Kwon H et al.[79]). **(D)** Examples of BL imaging using smartphones. BL reaction of paper-based microfluidics is presented in the upper panel.[97] To achieve cellular resolution smartphone should be adapted with the addition of fiber faceplate (lower panel).[99] With permission of ACS publications. Partially created with BioRender.com.

h-Pivaloyl-FMZ and Boc-FMZ.[74] With these two substrates, the free furimazine is slowly released by the deprotection process and can then react with luciferase inside cells.[75] Caging is also applied to D-luciferin by modification at the 6 hydroxyl (amino) group of D-luciferin, which, in turn, interferes with the reaction with luciferase and causes quenching of the BL emission. This strategy has been used for the generation of BLI systems, which is able to detect enzymatic activities, small bioactive molecules, or specific uptake, upon release of D-Luciferin, which can, in turn, be free to react and generate BL emission in a proportional manner. Caged D-luciferin substrates have been developed, for example, to measure activity of caspases, uptake of glucose and peptides.[76,77] Noteworthy, the enzymes or small molecules can be imaged at a desired time and location in living cells.

Moreover, in analogy to marine-derived enzymes, bacterial luciferases can also emit blue photons (λmax ~490 nm).[78] The operon, which consists of six major genes for the synthesis of the luciferase and the substrate, can be entirely inserted in the genome of the heterologous organism.[72] The independence from exogenous administration of luciferin renders their use particularly interesting, even though the photon yield of such system is low. Gregor and colleagues designed the iLUx operon with improved brightness and codon optimized it for expression in mammalian cells.[79,80] With this approach, it was possible to achieve imaging of BL signals from single mammalian cells.

3.2 | Compatibility with different cell sources and imaging/microscopy technologies

Detection of BL as readout of in vitro assays is generally performed using photomultiplier tubes inside luminometers, while imaging of luminescence requires the use of CCD cameras, mounted behind collection optics on top of a black box or in a microscope (Figure 2A and B). Despite many recent improvements, BL microscopy is still lagging behind in automation and 3D imaging capabilities. The light generated by single cells expressing luciferase reporters is defined, as "low-light" and conventional microscopes are inefficient at transmitting light from the sample to the detector. This means that imaging BL requires long exposure times (seconds to minutes). BL microscopy of promoter activity in single cells has been performed using ultra-low-light imaging cameras, such as liquid nitrogen-cooled CCD cameras, photon-counting CCD cameras, or image-intensifying CCD cameras.[81–86] Recently, electron-multiplying CCD (EM-CCD) camera, which yields higher sensitivity and image quality, was commercially released and

subsequently used for BL microscopy.[87,88] Apart from more sensitive detectors, improved microscopes for ultra-low-light imaging rely on modifications to the imaging lens and illumination and area of the imaging field and can reduce time of acquisitions for bright luminescent system (eg, Nanoluc/furimazine) to milliseconds.[89] Although BL microscopes are available on the market as the Olympus LV200 and the Atto Cellgraph, many other microscopes can be adapted for BL imaging. Most wide-field microscope setups equipped with highly sensitive CCD cameras can be adapted for BL detection with limited modifications (Figure 2A). For example, Tung and colleagues investigated which setup can convert a wide field microscope into a BL microscope, reaching signal to noise ratio of 130.[87] The recent work of Kim and colleagues describe how they successfully adapted their microscope for detection of BL and even radioluminescence in cells.[88,89] In the work of Gregor and colleagues, an EMCCD mounted on the microscope was upgraded with iXon EMCCD with a large field of view (512 × 512 pixels) and cooled at −100°C, to enhance sensitivity. Focal length was modified by exchange of the focusing lens. These settings allowed the authors to acquire the luminescence of the autologous BL imaging system within seconds. Microscopes adapted for BL detection allowed the imaging of highly dynamic events as protein–protein interactions in living cells using BRET, in a quantitative manner[88,90] and also to achieve organelle resolution. In addition, dual color imaging of BL from single cells is also possible. In fact, by addition of two dichroic mirrors, the light collimated by the objective lens could be divided into green and red light and reaches different areas of the CCD camera, after being focused by the imaging lens[51] (Figure 2C). This setting allowed imaging of dual gene expression in live cells and also in whole organisms, such as *Caenorhabditis elegans*.[58,91] In a recent work of Shan et al., visualization of molecular rhythms in subtypes of master clock neurons expressing two different luciferases in mouse brain was achieved.[92]

Finally, also sequential fluorescence-BL microscopy is now possible. Luciferins are fluorescent molecules that generate background if present in culture medium. In conventional assays, this confounds the simultaneous observation of fluorescence and BL. However, by selecting optical filter sets which consider the spectrum of emission of the fluorescence and BL reporters, together with luciferin concentrations of less than 100 micromolar, allowed Goda and colleagues to successfully image firefly luciferase and GFP in the same sample. This exciting achievement allowed the unique combination of the analysis of gene expression together with upstream signaling in living single cells.[93] Interestingly, the availability of novel photoactivatable luciferins may open up more possibilities for combining BL and fluorescence microscopy.[94]

3.3 | Interfacing bioluminescence imaging and organ-on-a-chip systems

In general, a suitable adaptor allows for on-chip platforms to fit into a wide-field inverted microscope. However, to monitor cell status while keeping a stable physiological temperature and constant 5% CO_2 with less disturbance to the system, compact microscopes that fit into the incubator have been developed. In addition, miniature microscopes have recently delivered enough sensitivity to detect BL, as in the case of the miniscope (BLmini).[95] This miniscope weighs only 2.5 g and displays an incorporated CMOS sensor. The authors successfully imaged Nanoluc BL dynamics in vivo, in the brain of mice, although with limited spatial resolution. Nevertheless, improved versions of this setup are expected to be developed in the near future for detection of signals in advanced in vitro platforms such as organs-on-chips. On the other hand, even smartphones can be nowadays simply adapted to image BL from noncell-based assays performed in paper microfluidic device[96,97] but also from single cells.[98] The CMOS camera of many smartphones can be interfaced with the organ-on-a-chip setup, using a detachable objective lens. In the work of Hattori and colleagues, imaging of HeLa cells cultured in a 35 mm dish and transiently expressing either NanoLuc or any colors of Nanolantern family of proteins (cyan, green, yellow, orange, or red) was possible, generating images of single cells of different colors.[99] This type of innovative work can be combined with organs-on-chips, opening new avenues for multiplex detection of simultaneous cell readouts, particularly interesting to study cell interactions in complex coculture systems.

4 | FUTURE DIRECTIONS AND CONCLUDING REMARKS

The main advantage of BL imaging relies in the unique high signal to noise ratio and the avoidance of phototoxicity/photobleaching, in comparison to fluorescence-based approaches. In last decade, scientist have developed increasing efforts into the generation of novel BL tools, to ultimately further the increment sensitivity of detection and multiplexing ability. This temporary shift of attention from hardware development may have resulted in the current limited adoption and wide use of BL imaging for organs-on-chips, despite its many advantages. It is generally foreseen that future directions on the development of this promising imaging method is the implementation of 3D, since currently all available BL analysis remains in a 2D format. This transition will greatly depend on future development of mathematical reconstruction of signals. Advancements in the field are also expected to include

further exploiting BRET probes, circumventing current technical issues such as photobleaching. Considering the availability of novel cheap, sensitive and miniaturized imaging platforms, the authors are confident that the integration of BL and its potential combination with fluorescence will flourish within the next decade. This promising marriage between BL imaging and organs-on-chips is expected increase the pace and efficacy of sensing key biological features, with the promise to enhance our understanding of human diseases, drug development and patient-specific therapy screening, in a cost-effective and straightforward manner.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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