luminicell

In vivo, long-term monitoring of transplanted stem cells using the Cellaris™ 670 fluorescent probe

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Abstract

Stem-cell transplantation, a promising direction for regenerative medicine, benefits from a means of tracking these cells over an extended period. Transduction of reporter genes into cells remains popular, but is labour and time intensive. Presented herein is the labelling and monitoring of adipose tissue-derived stem cells for up to 42 days after transplantation with a bio-compatible, 30-nm Cellaris™ 670 (Luminicell) nanoparticulate fluorescent probe. The solution is characterized by its exceptional brightness, photostability and resilience against various biological processes, ultimately yielding strong signal longevity. The extended duration of its tracking ability is unprecedented, compared with other exogeneous fluorophores, which represents a leap forward in simple stem-cell monitoring, unveiling its potential in other applications.

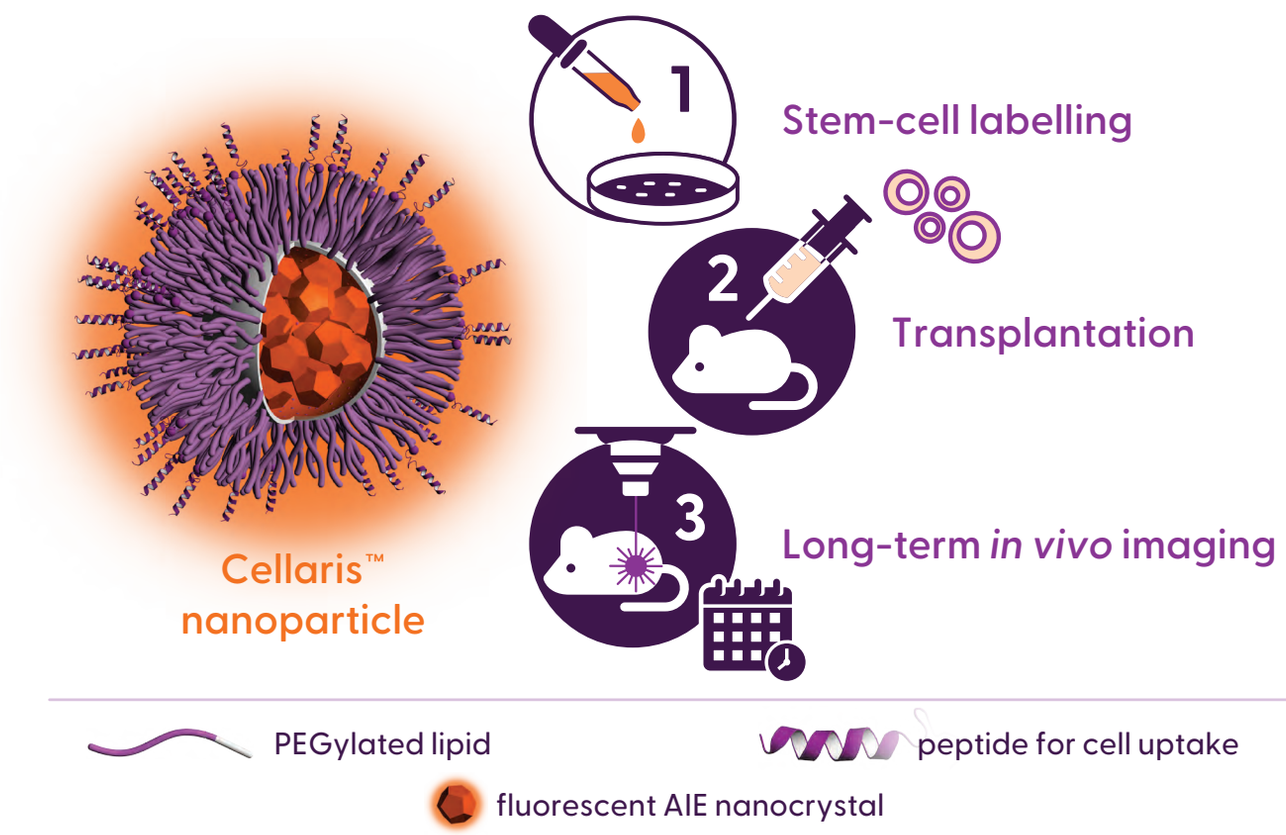

Figure 1. Processes for fluorescent labelling of stem cells and long-term *in vivo* tracking with a Cellaris nanoparticle, equipped with peptides for cell uptake.

Introduction

Understanding and monitoring the long-term distribution as well as the fate of transplanted cells *in vivo* are critical for advancing cell-based therapies. Traditionally, gene-editing processes, such as the use of luciferase or green fluorescent protein (GFP) reporter genes, have been employed for luminescent cellular labels. These methodologies often entail complex and time-consuming processes.

For decades, fluorophores have been used as widespread tools for cellular staining or labelling cells *in vitro* / *ex vivo* / short-term *in vivo* cases. The pursuit of *in vivo* fluorescent solutions for long-term imaging persists as a significant challenge. Most fluorophores are susceptible to photobleaching, drastically decreasing signals upon prolonged or repeated irradiation, especially from a laser. While quantum dots (QDs) may exhibit high photostability, they are composed of heavy metals, which may pose toxicity risks for the organism being studied, rendering them unsuitable for long-term *in vivo* application. Were fluorophores to offer simultaneous benefits of high labelling efficiency, brightness, photostability, biocompatibility and resilience to labelling and cellular processes, they would be of considerable utility in simpler labelling strategies than gene editing for long-term *in vivo* imaging (Figure 1).

Cellaris is an organic nanoparticle-based fluorescent platform technology with dyes, which possess a unique aggregation-induced emission (AIE) feature. Unlike conventional molecular dyes, which optically quench when aggregated, Cellaris contains fluorophores whose emissions are conversely enhanced. When packed and encapsulated into the core of nanoparticle, especially high fluorescence intensities result, wherein biocompatibility can be preserved and cell uptake properties tailored.[1] Cellaris is designed to provide simultaneous benefits of biocompatibility, bright fluorescence, outstanding photostability and high signal longevity, enabling live cell fluorescence labelling for longitudinal studies, especially for long-term *in vivo* imaging.[2] A variety of solutions at different wavelengths are available. Cellaris 670, with 30-nm diameter, has fluorescence emission similar to other far-red options such as Cy5, Cy5.5, Alexa Fluor 647/660/680, but excited with blue or green light sources (Ex 506 nm, Em 670 nm).

Experimental

Cell Isolation & Culture

1. Adipose tissue derived stem cells (ADSCs) were isolated from the abdominal and inguinal adipose tissue of 8 to 12-week-old male mice.
2. The isolated cells were subcultured *in vitro* with complete growth medium (α-Minimum Essential Medium with 20% FBS and 1% penicillin-streptomycin) and placed in a humidified incubator containing 5% CO_2.
3. The 3rd passage of ADSCs were used for labelling and imaging.

Cell Labelling & *In Vitro* Imaging

1. The ADSCs were seeded in an appropriate vessel, as required by application.
2. The medium was removed when the cells reached 80% confluence. Cells were subsequently washed with sterile 1× PBS.
3. To prepare the labelling solution (1 nM working concentration), the stock Cellaris solution (200 nM) was diluted with fresh growth medium and mixed by vortex. Other cell-tracking dyes (PKH26, QD655) were used at their respective manufacturer-recommended concentrations (2 µM, 1 nM, respectively) in separate treatments of different cell aliquots.
4. The labelling solution was added into cells, which were then incubated in a cell incubator (37 °C, 4 h).
5. Adherent cells were washed twice with growth medium.
6. The labelled cells were subcultured and imaged using a confocal laser scanning microscope (TSC SP8, Leica) with excitation at 543 nm and signal collection from 560 to 800 nm. The fluorescence intensities of the labelled cells were evaluated by a flow cytometer (Becton Dickinson), with excitation at 488 nm and emission collected by 680/20 nm bandpass filter.

Cell Transplantation & *In Vivo* Tracking

1. The ischemic hind limb model was established on 8-week-old female BALB/c mice (n=8).
2. ADSCs (1×10^6), labelled with Cellaris 670, were encapsulated in Matrigel (30 µL per mouse).
3. The prepared ADSC-containing Matrigel was injected intramuscularly into the ischemic hind limb of each mouse.
4. Mice were anaesthetized and imaged on 1, 7, 15, 25 and 42 days after injection, respectively. Fluorescence imaging was performed on a Maestro EX imaging system (Cambridge Research & Instrumentation) with conditions: excitation: 523 nm, emission : 560-900 nm (10-nm step, 200-ms exposure time). For bioluminescence imaging, D-Luciferin (150 mg/kg) was injected intraperitoneally into the same mice used for fluorescence imaging, and the mice were imaged with an IVIS Lumina II (Revvity, formerly Xenogen), receiving 1-s to 5-min scans.

Results & Discussion

In Vitro Cell Imaging

The *in vitro* cell-labelling efficiency and brightness of Cellaris 670 were investigated and benchmarked against two popular, commercial cell trackers: PKH26, a cell membrane dye and QD655, a quantum dot label.

After labelling with different cell tracker products separately, ADSCs were subcultured from 1 to 5 days. After 1 day of subculture, fluorescence signals were observed from all labelled ADSCs under confocal microscopy (Figure 2), and the Cellaris-labelled ADSCs observed as brightest. After 5 days, only weak fluorescence signals were detected in PKH26- and QD655-labelled cells, while the fluorescence remained significantly brighter for the Cellaris 670-labelled cells.

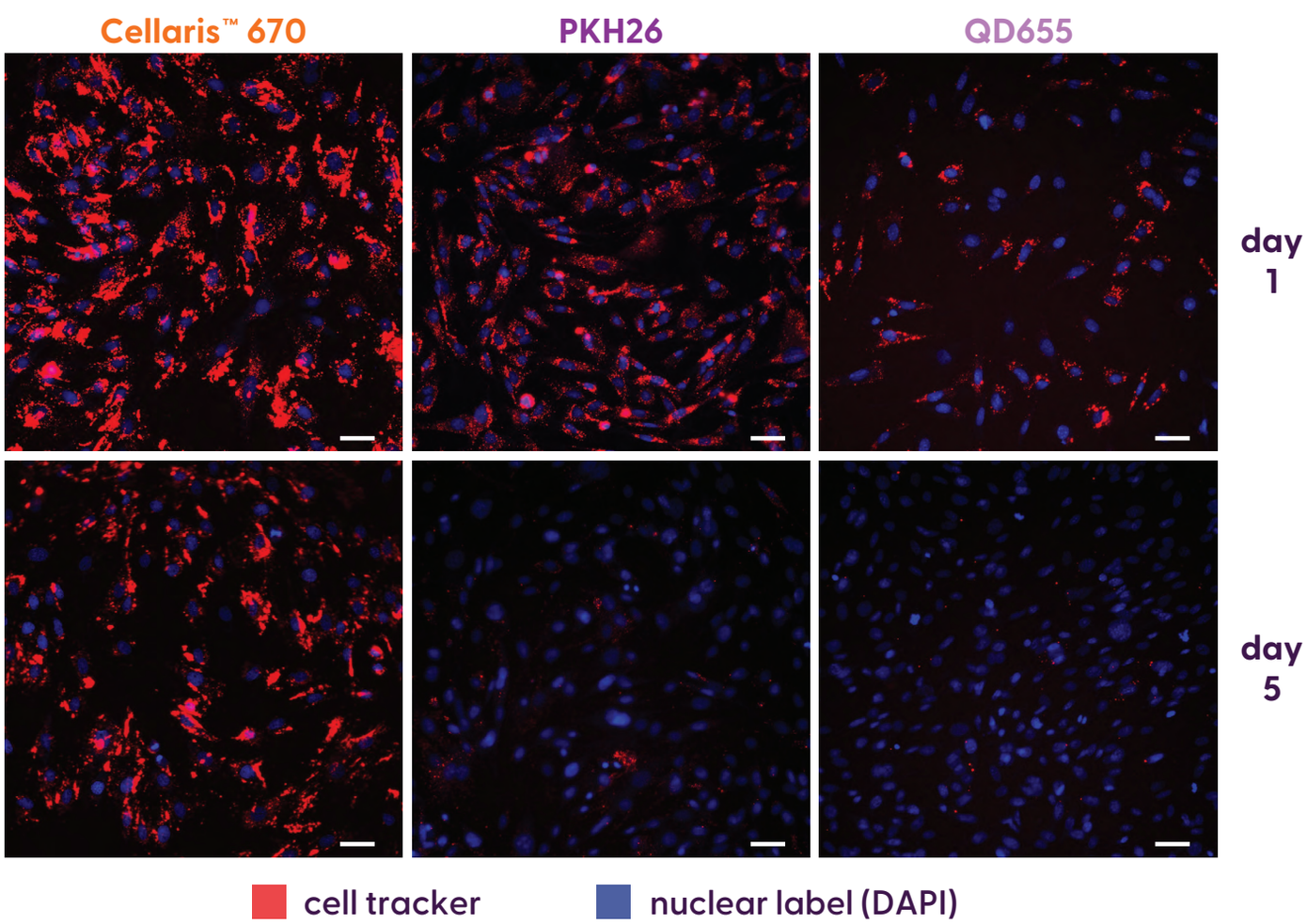

Figure 2. Confocal images (cell tracker: excitation 543 nm; emission 560-800 nm) of ADSCs, labelled with Cellaris 670 (1 nM), PKH26 (2 µM), & QD655 (1 nM) after having been subcultured *in vitro* for 1 & 5 days, respectively. Scale bar: 25 µm.

The cell labelling rate and the brightness and signal longevity of Cellaris 670-labelled single cells were measured quantitatively using flow cytometry (Figure 3). Measured after 1 day, Cellaris 670 exhibited high cell-labelling efficiency (98.5%) with comparable fluorescence intensity to PKH26 (97.5%), both of which are higher than QD655 (80.6%). On Day 5, retention of Cellaris 670 by ADSCs remains significantly higher (92.5%), even after multiple cell divisions, than PKH26- and QD655-labelled ADSCs (43.9% and 12.4% respectively), indicating photobleaching or clearance of the dyes. The results agree with the previous observations from confocal imaging, where Cellaris 670 exhibited higher brightness and greater signal longevity.

In Vivo Cell Tracking

The *in vivo* tracking ability of Cellaris 670 in stem cells has been examined on a hind-limb ischemia model. ADSCs, isolated in this study, are from the abdominal and inguinal adipose tissue of 8-12 week-old FVB-Luc-GFP transgenic mice, which express both luciferase and GFP (not used in this study).[2] The *in vivo* biodistribution of ADSCs in mice imaged with bioluminescence serves as a reference for the performance of Cellaris 670.

While after nearly 1.5 months, only some of the transplanted cells remained viable, shown by the reduction in Luciferase bioluminescence. The time-dependent fluorescence of the Cellaris 670-labelled ADSCs (Figure 4) generally correlate with the bioluminescence of Luciferase across the whole study and were still distinguishable at day 42. This establishes that Cellaris 670 is capable of precisely reporting of the distribution of transplanted ADSCs *in vivo*. Visualisation of fate of the transplanted stem cells, such as migration and differentiation, is presented elsewhere.[2] It has been demonstrated that the fluorescence of Cellaris is sufficiently bright and stable to be used as an alternative strategy to the widely accepted, Luciferase gold standard. Cellaris, moreover, offers the opportunity to investigate cell transplantation without gene modification, more simply, faster and with less cost. Studying stem cells with this method is of particular significance due to their poor transfection efficiency and concerns over altering the result from an unmodified organism.

Summary

The Cellaris 670 nanoparticle solution has been applied for long-term *in vivo* fluorescence tracking of transplanted stem cells. With its ultra-bright, far-red emission, coupled with extended signal longevity and biocompatibility,

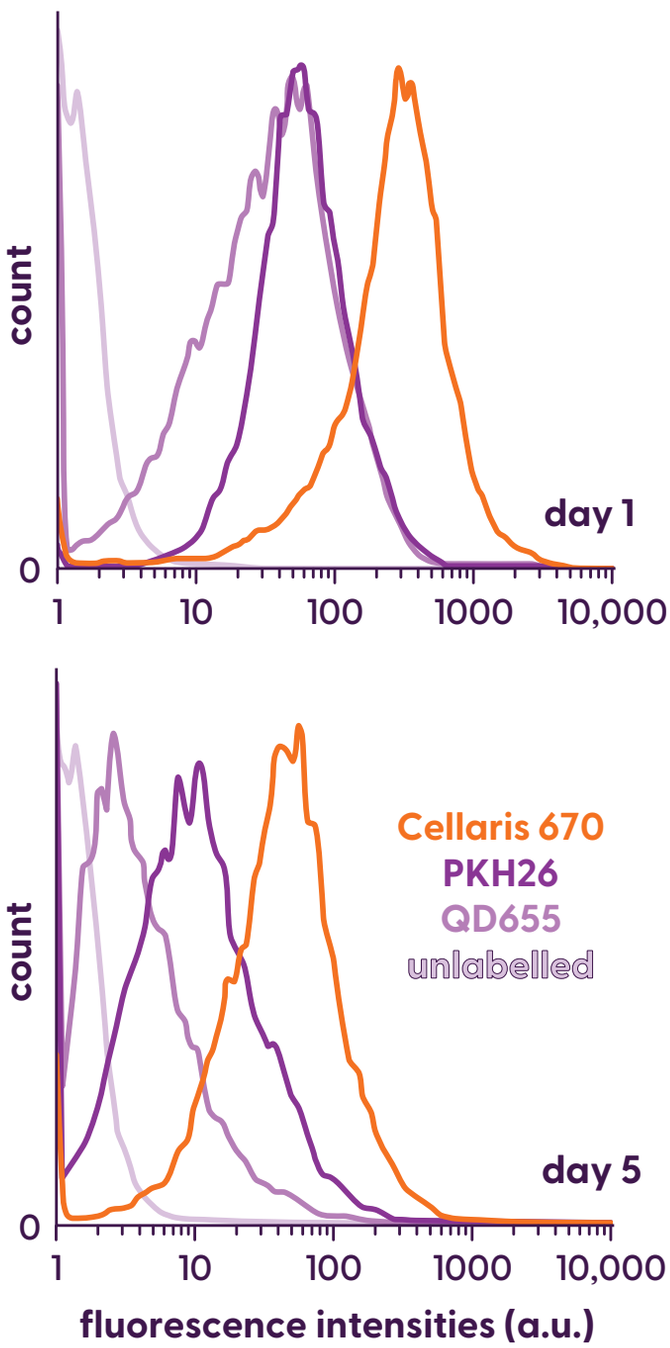

Figure 3. Flow cytometry (excitation 488 nm; emission 680/20 nm, band pass) results of ADSCs on day 1 and day 5 after labelling with Cellaris 670 (1 nM, orange), PKH26 (2 µM) and QD655 (1 nM). Unlabelled ADSCs are shown as negative control (light violet).

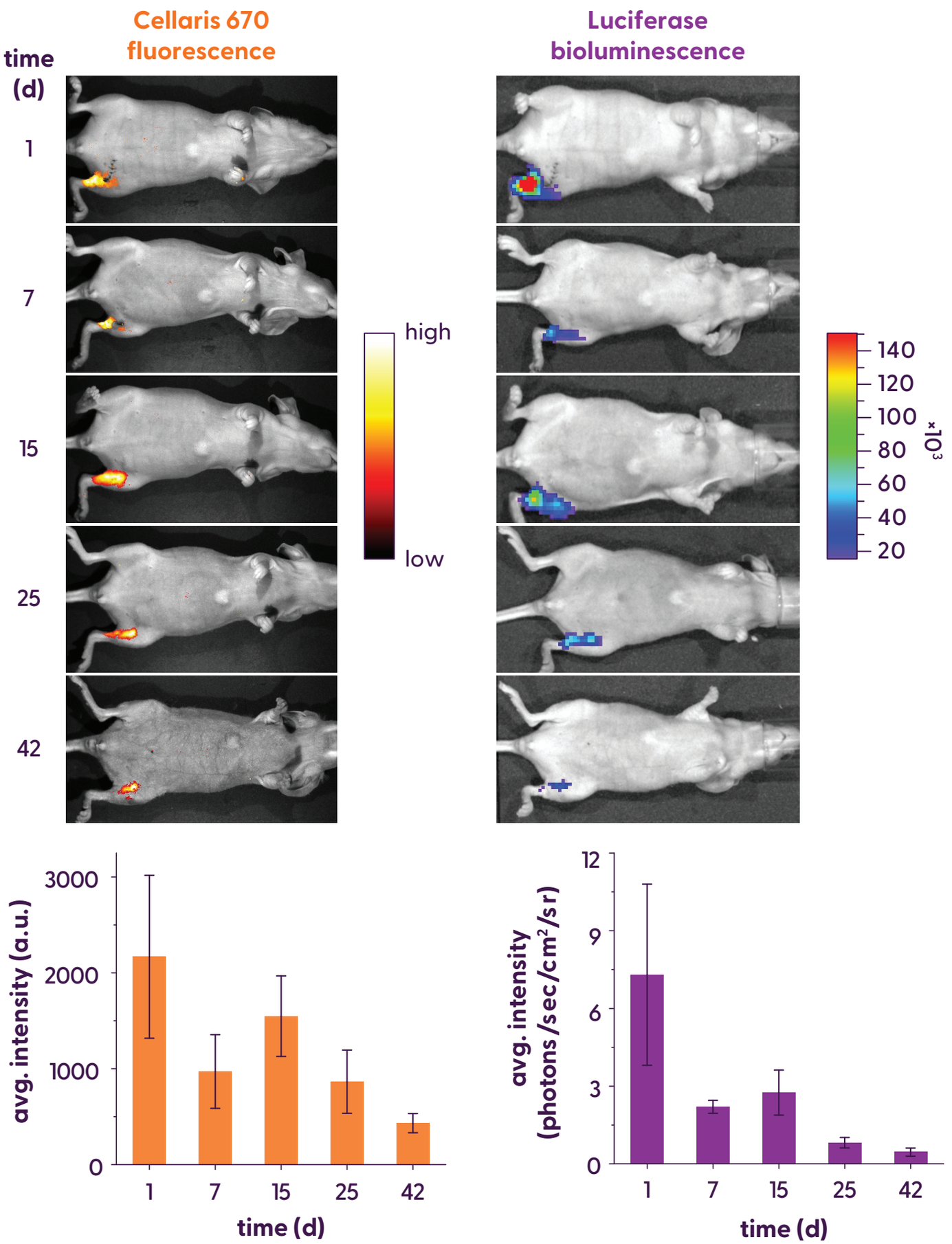

Figure 4. Representative time-dependent *in vivo* fluorescence and bioluminescence images of the ischemic hind limb-bearing mouse that was intramuscularly injected with Cellaris 670-labelled ADSCs, and the corresponding fluorescence and bioluminescence intensity changes in the region of the interest, averaged across 8 mice.

it can be seen as a prospective tool for long-term monitoring of the transplanted cells in living animals. Cellaris 670 is also capable of precise tracking of transplanted cells, which was validated against genetically-expressed Luciferase bioluminescence. We hope that the unprecedented duration (42 days) of its *in vivo* cell tracking properties with fluorescence will help to enable researchers to unravel the mysteries of cellular behaviour *in vivo*. Tracking durations may indeed be longer. Apart from stem-cell applications[3-6], Cellaris has also been successfully applied to long-term *in vivo* monitoring of other types of transplanted cells, including but not limited to xenogeneic cells for tumor immunotherapy[7,8] and human embryonic stem cell-derived neurons.[9] We foresee more contributions of Cellaris to the progress of regenerative medicine and therapeutic interventions.

Acknowledgments

The reported results are drawn mainly from previously published results[2] with permission from the authors and copyright permission, given by the American Chemical Society.

Cover image

The stylised header image shows Cellaris 670 loaded into HeLa cells.

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Cell-labelling kits

product name[1]	Ex (nm)	product code	configuration				
			kit volume[2] (µL) code			surface	
						code	description
Cellaris 506	355	LCTC-506	100	250	500	01	CPP[3]
Cellaris 540	423	LCTC-540					
Cellaris 670	506	LCTC-670					
Cellaris 810	635	LCTC-810					
Cellaris 1010	725	LCTC-1010					

Notes

1. Product names correspond to their respective emission wavelengths (nm) in water. Excitation (Ex) maxima are listed separately.
2. Kit volumes of 100-, 250- & 500-µL are typically suitable for 20, 50 & 100 tests, respectively.
3. Nanoparticle surfaces are PEGylated, and surface conjugated with cell-penetrating peptides (CPP) for cell uptake. Other surface chemistries are available upon request.

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