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A proof-of-concept study to investigate the radiolabelling of human mesenchymal and hematopoietic stem cells with [^{89}Zr]Zr-Df-Bz-NCS

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Abstract

Background: The transplantation of hematopoietic stem and progenitor cells (HSPCs) or mesenchymal stromal/stem cells (MSCs) for the treatment of a wide variety of diseases has been studied extensively. A challenge with cell-based therapies is that migration to and retention at the target site is often difficult to monitor and quantify. Zirconium-89 (^{89}Zr) is a positron-emitting radionuclide with a half-life of 3.3 days, which allows long-term cell tracking. Para-isothiocyanatobenzyl-desferrioxamine B (Df-Bz-NCS) is the chelating agent of choice for ^{89}Zr-cell surface labelling. We utilised a shortened labelling method, thereby avoiding a 30–60-min incubation step for [^{89}Zr]Zr-Df-Bz-NCS chelation, to radiolabel HSPCs and MSCs with zirconium-89.

Results: Three ^{89}Zr-MSC labelling attempts were performed. High labelling efficiencies (81.30 and 87.30%) and relatively good labelling yields (59.59 and 67.00%) were achieved with the use of a relatively larger number of MSCs (4.425 and 3.855 million, respectively). There was no significant decrease in MSC viability after ^{89}Zr-labeling ($p = 0.31$). This labelling method was also translatable to prepare ^{89}Zr-HSPC; preliminary data from one preparation indicated high ^{89}Zr-HSPC labelling efficiency (88.20%) and labelling yield (71.06%), as well as good HSPC viability after labelling (68.65%).

Conclusions: Successful ^{89}Zr-MSC and ^{89}Zr-HSPC labelling was achieved, which underlines the prospects for in vivo cell tracking studies with positron emission tomography. In vitro investigations with larger sample sizes and preclinical studies are recommended.

Keywords: Mesenchymal stem cells (MSCs), Hematopoietic stem and progenitor cells (HSPCs), Cell labelling, In vivo cell tracking, Zirconium-89, Positron emission tomography

Background

There has been increasing interest and research in stem cell-based therapies over the last two decades. Stem cell therapy has the potential to directly treat certain diseases through the replacement of diseased or impaired cells, which highlights its application

in regenerative medicine (Ghasroldasht et al. 2022; El-Kadiry et al. 2021; Lee and Hong 2020; Rajendran et al. 2020; Brown et al. 2019). Possibly the most commonly known cell-based therapy in clinical practice is the transplantation of human adult hematopoietic stem and progenitor cells (HSPCs), via intravenous infusion, for specific blood diseases, e.g. leukaemia and lymphoma (El-Kadiry et al. 2021; Lee and Hong 2020; Brown et al. 2019). HSPCs are mainly derived from bone marrow, but can also be sourced from peripheral blood and umbilical cord blood, and can differentiate into mature blood cells. Their role in the treatment of liver diseases and solid cancers, amongst various other diseases, are currently topics of interest (El-Kadiry et al. 2021; Lee and Hong 2020).

The use of mesenchymal stromal/stem cells (MSCs) as a cell-based therapy has recently been explored for a number of different diseases and conditions (Rajendran et al. 2020; Pradhan et al. 2022; Wang et al. 2021; Rajarshi et al. 2020; Saeedi et al. 2019). MSCs are multipotent adult stem cells mainly sourced from bone marrow, adipose tissue, umbilical cord and dental pulp that can differentiate into any mesoderm-derived cell type, including skeletal, cartilage, muscle, cardiac, and vascular cells (El-Kadiry et al. 2021; Wang et al. 2021; Saeedi et al. 2019; Ferretti and Hadjantonakis 2019). MSCs are also involved in the recruitment of macrophages, lymphocytes, and other immune cells to sites of disease or injury to promote healing. They have been the subject of numerous clinical studies for the potential treatment of a variety of immune-related diseases, such as graft-vs-host disease, autoimmune diseases (e.g. irritable bowel syndrome, multiple sclerosis, rheumatoid arthritis and type 1 diabetes mellitus), osteoarthritis, liver cirrhosis and even COVID-19 (Brown et al. 2019; Wang et al. 2021; Rajarshi et al. 2020; Saeedi et al. 2019; Jasim et al. 2022; Golchin et al. 2020). The regenerative capability of MSCs led to the investigation of their potential use in spinal cord injuries (Cofano et al. 2019) and neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease (Brown et al. 2019; Pradhan et al. 2022). Many preclinical studies have been conducted utilising MSCs as treatment for different cancers (Rajendran et al. 2020; Saeedi et al. 2019).

A challenge with cell-based therapies is that migration to and retention at the target site is not guaranteed and is often difficult to monitor. This may lead to decreased efficiency of these expensive and potentially life-saving therapies in the clinical setting. Additionally, it can result in poor understanding of their in vivo behaviour and efficacy in preclinical investigations, causing delays in human studies (Friberger et al. 2021; Lechermann et al. 2021). In vivo cell tracking with Nuclear Medicine imaging offers a sensitive and non-invasive solution to assess the homing and retention of transplanted cells to their desired site of action (Rajendran et al. 2020; Friberger et al. 2021; Lechermann et al. 2021). Zirconium-89 (^{89}Zr) is a positron-emitting radionuclide that has gained much interest over the last few years as a direct cell-labelling agent, due to its half-life of 3.3 days, which matches well with the need for long-term cell tracking by way of PET imaging (Rajendran et al. 2020; Friberger et al. 2021; Lechermann et al. 2021; VanBrocklin 2021).

Literature reports on the ^{89}Zr-radiolabelling of a variety of cells aiming at in vivo cell tracking, including MSCs, HSPCs, chimeric antigen receptor (CAR) T cells, dendritic cells, natural killer cells, hepatocytes, cardiopoietic stem cells, and leukocytes, with various degrees of success (Friberger et al. 2021; Kahts et al. 2023; Bansal et al. 2022, 2015; Wang et al. 2022; Yoon et al. 2020; Pantin et al. 2015; Sato et al. 2015). Two strategies

for direct cell labelling with zirconium-89 are proposed: (a) a lipophilic, neutral [^{89}Zr]Zr-oxine complex, which is capable of crossing the cell membrane and dissociates once inside the cell where the zirconium-89 binds to various cytoplasmic components and the oxine component leaves the cell (Friberger et al. 2021; Bansal et al. 2020) and (b) cell membrane ^{89}Zr-labelling using bifunctional chelating agents such as para-isothiocyanatobenzyl-desferrioxamine B (Df-Bz-NCS) to harbour the ^{89}Zr-activity as was first reported by Bansal et al. (2015). The Df-Bz-NCS binds covalently to exposed amine groups on cell surface proteins, allowing cell surface labelling with [^{89}Zr]Zr-Df-Bz-NCS, instead of intracellular ^{89}Zr-entrapment (Lechermann et al. 2021; Bansal et al. 2020).

We recently reported on an optimised, and significantly shortened labelling method providing excellent coverage of leukocyte cell surfaces with [^{89}Zr]Zr-Df-Bz-NCS (Kahts et al. 2023). This method circumvents a lengthy 30–60-min incubation step for [^{89}Zr]Zr-Df-Bz-NCS conjugation.

This study aims to prove whether HSPCs and adipose tissue-derived MSCs can be radiolabelled with [^{89}Zr]Zr-Df-Bz-NCS using the abovementioned approach without any major disadvantages or challenges to their cellular viability.

Methods

This proof-of-concept study was analytical and experimental. The study aimed to prove whether HSPCs and adipose tissue-derived MSCs can be radiolabelled with [^{89}Zr]Zr-Df-Bz-NCS using our recently reported optimised, and significantly shortened labelling method, without any major disadvantages or challenges to cellular viability. Radiolabelling was performed at the South African Nuclear Energy Corporation (Necsa) in Pelindaba. Labelling success was determined by measuring labelling efficiency and viability of the ^{89}Zr-labelled HSPCs or MSCs.

Stem cell material

All necessary reagents for the stem cell work, along with two flasks of thawed adipose-derived human MSCs and one flask of human HSPCs were supplied by the Institute for Cellular and Molecular Medicine (ICMM), South African Medical Research Council (SAMRC), Extramural Unit for Stem Cell Research and Therapy, University of Pretoria. This followed the acquisition of ethical approval for this study from the institutional review board at the Sefako Makgatho Health Sciences University (Ref no. SMUREC/P/21/2017: PG) and informed consent to use cryopreserved stem cells from healthy donors.

Preparation of adipose-derived MSCs

Lipoaspirates were obtained from healthy donors undergoing elective liposuction procedures. The stromal vascular fraction was isolated from lipoaspirates using an adaptation of a previously described method (Vollenstee et al. 2016). Adipose-derived MSCs were counted and seeded in complete growth medium [CGM: Dulbecco's Modified Eagles Medium (DMEM, Gibco, Grand Island NY, USA); 10% human platelet lysate (HPL); 2% penicillin/streptomycin (pen/strep, Gibco); 0.012% Heparin (Gibco)] at a density of 5000 cells per cm^2 in T75 flasks. Adipose-derived MSCs were removed from cryopreservation

and cultured in CGM until confluent. MSCs at passage 3 were used for the labelling experiments.

The flask containing the adherent MSCs in CGM was transferred from the CO_2 incubator to the laminar flow cabinet and the growth medium was aspirated. TrypLE™ (3 mL; Gibco) were added to the cells and incubated for 10 min at 37 °C. After incubation, the cells were dislodged gently and 6 mL of CGM was added to the flask to neutralize the TrypLE activity. The cell suspension was transferred to a clean 50-mL centrifuge tube (Greiner Bio-One, Kremsmünster, Austria) and the flask was rinsed with 5 mL phosphate buffered saline (PBS; containing 2% pen/strep). The cell suspension was centrifuged at 300 × *g* for 5 min (Digicen 21 R; Orto Alresa, Madrid, Spain) followed by removal of the supernatant and resuspension of the cell pellet in 1 mL DMEM. The cells were then incubated at 37 °C, while the [^{89}Zr]Zr-Df-Bz-NCS complexation was performed. The incubation period depended on the time it took to perform [^{89}Zr]Zr-Df-Bz-NCS complexation.

Isolation and preparation of HSPCs

CD34 + HSPCs were isolated (positive selection) from a cryopreserved leukapheresis product using the human CD34 MicroBead Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions. The isolated CD34 + HSPCs were transferred to a T75 culture flask containing 7 mL complete Roswell Park Memorial Institute medium [RPMI basal medium (Gibco, Grand Island NY, USA) supplemented with 10% foetal bovine serum (Gibco, Grand Island NY, USA)] and placed in a 37 °C/5% CO_2 water-jacketed incubator overnight (Thermo Fischer Scientific, Waltham, USA). The HSPC cell suspension was transferred to a 50-mL centrifuge tube the next day for ^{89}Zr-labelling.

HSPCs and MSCs will be referred to as stem cells from hereon in the description of the ^{89}Zr-labelling process. The same ^{89}Zr-labelling method was used for both stem cell types.

[^{89}Zr]Zr-Df-Bz-NCS radio-complexation

[^{89}Zr]Zr-oxalate was provided by Necsa (Pelindaba, South Africa) through collaboration with the Korea Atomic Energy Research Institute (KAERI; Daejeon, South Korea). For each labelling attempt, the volume of [^{89}Zr]Zr-oxalate containing 15–26 MBq was determined, added to a 15-mL Falcon tube and neutralised to a pH of 6–8 with a sodium bicarbonate solution (7.5%, sterile-filtered, BioReagent, suitable for cell culture, Sigma-Aldrich, St Louis, MO, USA). The total reaction volume was recorded.

Our recently published ^{89}Z-radiolabelling approach for cells was utilized (Kahts et al. 2023). Briefly, 1 mg Df-Bz-NCS (Macrocyclics, Inc., Plano, Texas, USA) was weighed off in a 2-mL Eppendorf tube (Greiner Bio-One, Kremsmünster, Austria) and mixed with 13 µL dimethyl sulfoxide (DMSO; Life Technologies Corporation, Carlsbad, CA, USA). This mixture was centrifuged at 1000 rpm for 1 min to form a pellet, after which the supernatant containing the dissolved Df-Bz-NCS was gently removed without disturbing the pellet and added to the neutralized [^{89}Zr]Zr^{4+}. PBS (915 µL) was added to the [^{89}Zr]Zr-Df-Bz-NCS solution.

Radiolabelling of stem cells with [^{89}Zr]Zr-Df-Bz-NCS

The stem cell suspension was transferred from the CO_2 incubator to the LAF. The whole stem cell suspension prepared for the specific day was utilised for the radiolabelling experiment, independent of the number of cells prepared, as this proof-of-concept study exclusively aimed to investigate whether our modified, shortened method can be used to successfully radiolabel stem cells with zirconium-89, and did not intend to investigate the effect of different cell counts on the resultant labelling efficiency. The cells were washed with 5 mL PBS via centrifugation at 300×g for 5 min, after which the cell pellet was gently dispersed in 750 µL PBS for labelling. The washed cells were then added to the [^{89}Zr]Zr-Df-Bz-NCS solution and the radioactivity measured and recorded using a Capintec CRC-15R dose calibrator (Florham Park, NJ, USA). The mixture was incubated at 37 °C for 45 min. After incubation, the labelled cells were washed twice with 5 mL PBS through centrifugation at 300×*g* for 5 min and the supernatants combined in a clean 50-mL tube. Both the ^{89}Zr-activity per cell pellet and the ^{89}Zr-activity in the combined supernatants were measured in the dose calibrator and recorded to calculate the labelling efficiency. The labelling efficiency was expressed as the percentage ratio between the radioactivity associated with the stem cells related to the total radioactivity (Gawne et al. 2022). The washed ^{89}Zr-labelled stem cells were resuspended in 3 mL PBS to allow viability testing. The ^{89}Zr-activity incorporated in the final cell suspension was also expressed as a percentage of the ^{89}Zr-activity at the start of labelling to obtain the labelling yield (Coenen et al. 2019).

Stem cell viability

Before and after radiolabelling, a cell viability test was performed on MSC- or HSPC starting material and the radiolabelled stem cells, respectively. An automated Cellometer® K2 (Nexcelom Bioscience LLC, Lawrence, Massachusetts, MA, USA) cell count was performed as per manufacture's instruction. Briefly, a 20-µL sample of the washed stem cells suspended in 750 µL of PBS for labelling was stained with 20 µL AO/PI (Acridine Orange/Propidium Iodide) staining solution (Cellometer® AOPI Staining Solution, Cat#: CS2-0106-5ML; Nexcelom Bioscience LLC, Lawrence, Massachusetts, MA, USA). The stained sample was pipetted into a Cellometer® Cell Counting Chamber (SD100 Counting Chamber, Nexcelom Part #: CHT4-SD100-002; Nexcelom Bioscience LLC, Lawrence, Massachusetts, MA, USA), the chamber was inserted into the Cellometer® K2 and an automated viability assay was performed. The viability test was repeated immediately after ^{89}Zr-labelling of the stem cells and again 2 h after labelling for the successful labelling attempts.

Results

In this proof-of-concept study, three ^{89}Zr-MSC and one ^{89}Zr-HSPC labelling attempts were performed, limited by the number of stem cells supplied.

Table 1 Total reaction volume, MSC count and viability before labelling, ^{89}Zr-MSC labelling efficiency and yield (n = 3)

Experiment #	Total reaction volume (µL)	MSCs used for labelling (cells/750 µL)	MSC viability before labelling (%)	Labelling efficiency (%)	Labelling yield (%)
1	1705	4 425 000	85.80	81.30	59.59
2	1728	3 855 000	94.35	87.30	67.00
3	1768	194 250	62.20	23.00	19.46

[^{89}Zr]Zr-Df-Bz-NCS radiolabelling of MSCs

A summary of the total reaction volume, the number of MSCs available for labelling and their viability, as well as the labelling results (labelling efficiency and labelling yield) are presented in Table 1.

^{89}Zr-activities of 18.13 MBq, 25.79 MBq and 17.87 MBq were utilised for labelling experiments #1, #2 and #3, respectively. High labelling efficiencies (81.30 and 87.30%) and good radiolabelling yields (59.59 and 67.00%) were achieved for labelling experiments #1 and #2, which seemed to be the result of a large number of MSCs (4.425 and 3.855 million, respectively) with excellent viability (85.80 and 94.35%) available for ^{89}Zr-labelling. For labelling experiment #3, radiolabelling was challenged by the availability of a significantly smaller number of MSCs for radiolabelling (194 250 cells) with lower cell viability (62.20%). This setting resulted in sub-standard labelling efficiency (23.00%) and labelling yield (19.46%).

It is not surprising that higher ^{89}Zr-MSC labelling efficiencies were achieved with an increased number of MSCs used for labelling. The viability of the MSCs also seemed to proportionally affect the labelling efficiency. These results imply that a higher number of viable cells may result in increased labelling efficiencies.

Rajendran et al. (2020) reported that MSC numbers as low as 6250–25000 can effectively be detected with PET, due to the sensitivity and high resolution of this imaging modality, and the relatively high tolerance of MSCs to radiation. However, the results of this proof-of-concept study challenge the statement by Rajendran et al. (2020), as sufficient labelling efficiency of such a low number of cells do not seem probable. Further studies with larger sample sizes are needed to reliably prove this theory.

For all three experiments the tolerance of MSCs to radiation exposure, as described in literature, was tested through cell viability determination before and after ^{89}Zr-labelling (Fig. 1).

Despite only 3 experiments being performed, the results provided in Fig. 1 demonstrate no significant decrease in the relative MSC viability due to the procedures required for ^{89}Zr-labelling ($p = 0.31$). In fact, an apparent increase in MSC viability was observed after ^{89}Zr-labelling in labelling attempt 3, from 62.2 to 77.7%. This higher measured percentage of viable cells in the ^{89}Zr-labelled MSCs sample may be due to the removal of dead MSCs during the washing steps after ^{89}Zr-labelling. The rationale behind this statement is that dead cells, which were already present in the cell suspension before labelling, are generally fractured and therefore become less dense than viable cells, which means that these cells (whether radiolabelled or unlabelled) will separate from the viable cells during centrifugation into the resultant supernatant and will therefore not form

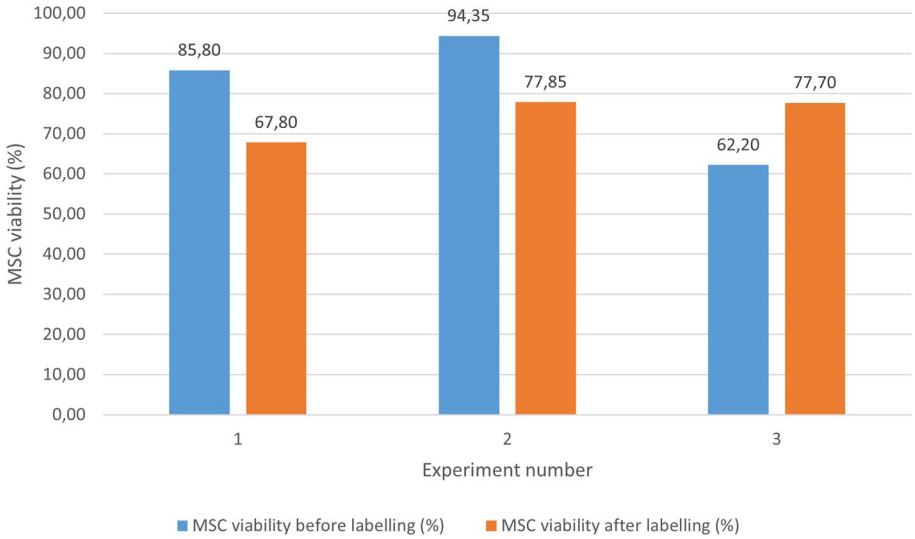

Fig. 1 MSC viability (%) before and immediately after ^{89}Zr-labelling (n = 3). The tolerance of MSCs to radiation exposure was tested through automated cell viability determination before and immediately after ^{89}Zr-labelling with the use of a Cellometer® K2. No significant decrease in the relative MSC viability due to the procedures required for ^{89}Zr-labelling was observed ($p = 0.31$)

part of the cell pellet formed during centrifugation. After ^{89}Zr-labelling and the washing steps that follow, there may therefore be a smaller number of MSCs present, but with a higher percentage of viability.

Viability testing of the ^{89}Zr-labelled MSCs for labelling attempts 1 and 2 were repeated 2 h after labelling. Interestingly, the ^{89}Zr-MSC viability for attempt 1 increased from 67.8% immediately after labelling to 81.7% at 2 h after labelling, while the viability for attempt 2 decreased from 77.9% immediately after labelling to 50.7% at 2 h post-labelling. To determine whether there was any further decrease in the viability of ^{89}Zr-MSCs, viability testing of the labelled cells from the second labelling attempt was repeated 24 h post-labelling and revealed increased viability of 58.2%. It is not clear from the current study results which factors affected the viability of the MSCs, even though radiation exposure of the cells can be ruled out as a suspect. Further studies are needed to assess and compare MSC viability from different donors and under different storage conditions.

Among the factors investigated to determine their effect on the ^{89}Zr-MSC labelling efficiency, the only other potential contributor identified was the reaction volume. From the data in Table 1 it can be observed that the lower reaction volumes generally yielded higher labelling efficiencies.

^{89}Zr-labelled HSPCs

Only one ^{89}Zr-HSPC labelling attempt was possible. However, successful radiolabelling was achieved, as summarised in Table 2.

A high ^{89}Zr-HSPC labelling efficiency (88.20%) and labelling yield (71.06%) were obtained, indicating successful labelling. The maintenance of HSPCs is slightly more challenging when compared to MSCs, which may explain the significantly low cell viability before labelling. However, after ^{89}Zr-labelling the viability increased to 68.65%. As

Table 2 ^{89}Zr-activity, HSPC count and viability before labelling, ^{89}Zr-HSPC viability, labelling efficiency and yield (n = 1)

^{89}Zr-activity (MBq)	HSPCs used for labelling (cells/750µL)	Cell viability before labelling (%)	Labelling Efficiency (%)	Labelling yield (%)	Cell viability after labelling (%)
20.202	1 057 500	22.40	88.20	71.06	68.65

explained previously, this phenomenon may be explained by the removal of dead HSPCs during the washing steps after completing the radiolabelling process which possibly resulted in a smaller total number of cells at the end of labelling, but likely contained a higher percentage of viable cells in the sample.

Discussion

Cell-based therapies using MSCs and HSPCs have gained major attention in both preclinical and clinical studies for the treatment of a variety of conditions, including immune-related diseases, autoimmune disorders, cardiovascular diseases, liver diseases, osteoarthritis, neurodegenerative conditions, spinal cord injuries, and various cancers (Rajendran et al. 2020; Brown et al. 2019; Pradhan et al. 2022; Wang et al. 2021; Rajarshi et al. 2020; Saeedi et al. 2019; Jasim et al. 2022; Golchin et al. 2020; Cofano et al. 2019; Bansal et al. 2022; Wolfs et al. 2015). To accurately evaluate the in vivo behaviour of transplanted stem cells in a noninvasive and highly sensitive manner, molecular imaging using PET can be employed to produce high quality three-dimensional images (Rajendran et al. 2020; Friberger et al. 2021; Lechermann et al. 2021). A PET radionuclide, with a relatively long half-life that correlates to the biological half-life of viable stem cells, is required for in vivo cell tracking. This fact has generated major interest in the use of zirconium-89 for the radiolabelling and resultant in vivo tracking of cells. The 78.4-h half-life of zirconium-89 allows accurate cell tracking for at least 7 days, with some resources even stating the possibility of a 3-week observation period (Rajendran et al. 2020; Friberger et al. 2021; Lechermann et al. 2021; VanBrocklin 2021; Bansal et al. 2022, 2020; Wang et al. 2022; Yoon et al. 2020; Gawne et al. 2022; Melendez-Alafort et al. 2023).

A detailed review of all major ^{89}Zr-cell labelling studies since 2015 was published by Melendez-Alafort et al. (2023). In summary, labelling efficiencies ranging from 13 to 75% for [^{89}Zr]Zr-oxine-labelled cells, and 30–79% for [^{89}Zr]Zr-Df-Bz-NCS-labelled cells, were reported, dependent on the type of cell labelled. If we only consider human stem cell labelling attempts, Bansal et al. (2015) achieved relatively low labelling efficiencies of 30–50% for the labelling of human MSCs with [^{89}Zr]Zr-DFO-NCS, with no significant difference in the cell viability before and after ^{89}Zr-labelling. ^{89}Zr-MSC labelling was repeated by Bansal et al. (2022) more recently, with an average labelling efficiency of 22.77 ± 5.02% (n = 3) and high MSC viability of approximately 90–95% reported. Bansal et al. (2020) also attempted [^{89}Zr]Zr-DFO-NCS-labelling of cardiopoietic stem cells and achieved labelling efficiencies ranging from 30 to 40%. They did not observe any changes in cell viability before and after labelling. Wang et al. (2022) labelled human bone marrow-derived MSCs with [^{89}Zr]Zr-oxine and obtained an average labelling efficiency of 52.6 ± 0.01%. They observed a negative correlation between the [^{89}Zr]Zr-oxine

activity used and cell viability, with the highest labelling efficiency (59.4%) and cell viability (84.9%) obtained when the lowest ^{89}Zr-activity was used for labelling. The differences in the effect of ^{89}Zr-activity on cell viability reported by these three studies are interesting and can possibly be explained by the position of the radiolabel relative to the cell. [^{89}Zr]Zr-oxine crosses the cell membrane and accumulates within the cell, while [^{89}Zr]Zr-Df-Bz-NCS binds to the primary amine groups on the cell surface (Friberger et al. 2021; Lechermann et al. 2021; Bansal et al. 2022, 2015, 2020). The increased distance of the radiolabel from sensitive intracellular components with the cell membrane labelling approach may suggest decreased radiation exposure and resultant effects of radiation on these components.

The results of the current study using our modified cell surface labelling approach illustrated successful ^{89}Zr-MSC and ^{89}Zr-HSPC labelling. Even with the small sample, much higher labelling efficiencies were achieved than those reported for ^{89}Zr-stem cell labelling in literature. We also found that the ^{89}Zr-MSC labelling efficiency may have been proportionally related to the number of stem cells used for radiolabelling.

Bansal et al. (2022) used a high concentration of 6 million MSCs suspended in 500 µL for labelling with a resultant average labelling efficiency of 22.77 ± 5.02% (n = 3). Our combined results for ^{89}Zr-MSC and ^{89}Zr-HSPC labelling illustrated an average labelling efficiency of 66.95 ± 24.36% (n = 4) utilising an average of approximately 2.4 million stem cells suspended in 750 µL for labelling—less than half of the number of stem cells utilised for labelling by Bansal et al. (2022). Only one labelling attempt resulted in a low labelling efficiency due to a very small number of stem cells available for labelling (only 194 250 MSCs). Similarly to other studies (Bansal et al. 2022, 2015, 2020), no significant decrease in the viability of MSCs and HSPCs were observed after ^{89}Zr-labelling.

The only other factor that seemed to affect the ^{89}Zr-stem cell labelling efficiencies and yields, was the reaction volume, which suggests that smaller reaction volumes will result in increased labelling efficiencies and yields. It is recommended that for future investigations, the washed stem cells be resuspended in a smaller volume for labelling (< 750 µL) and the [^{89}Zr]Zr-Df-Bz-NCS complex diluted with a smaller volume of PBS (< 915 µL) before labelling, to reduce the total reaction volume.

Clinical investigations of ^{89}Zr-labelled cells over the last five years were recently reviewed by Duvenhage et al. (2024). Only three human studies have been reported, which investigated the in vivo biodistribution and resultant dosimetry of [^{89}Zr]Zr-oxine-labelled autologous leukocytes in four healthy human volunteers, as well as PET/CT infection imaging with [^{89}Zr]Zr-Df-Bz-NCS-labelled mononuclear cells in seven patients. The possibility of longitudinal ^{89}Zr-PET imaging was demonstrated in these studies, with in vivo cell tracking being performed for up to 144 h post-administration of the radiolabelled cells. The promising results of these clinical studies warrant further investigations. However, the latter study reported a concerning challenge with the pulmonary trapping of administered ^{89}Zr-labelled cells, which needs to be addressed before further clinical studies can be planned. No human studies with ^{89}Zr-labelled stem cells have been reported to date.

Conclusions

This study investigated the radiolabelling of MSCs and HSPCs with zirconium-89, via Df-Bz-NCS as the bifunctional chelating agent, using our previously published ^{89}Zr-cell labelling procedure (Kahts et al. 2023). Our labelling method does not require the 30–60-min incubation step of zirconium-89 with the bifunctional chelate (Df-Bz-NCS) for [^{89}Zr]Zr-Df-Bz-NCS complex formation, which shortens the entire labelling process.

Successful ^{89}Zr-stem cell labelling was achieved with much higher labelling efficiencies than reported in other studies, as well as good viability of the ^{89}Zr-labelled stem cells. The only two factors that seemed to affect the labelling efficiency were the number of stem cells available for labelling, where an increased cell count resulted in increased labelling efficiency, and the total reaction volume, where decreased reaction volumes may result in higher labelling efficiencies. The limited number of radiolabelling experiments that were possible in this study limit the generalizability of the study results. It is, however, worth noting that some other studies reporting on stem cell labelling also utilised very small sample sizes (Bansal et al. 2022), similarly to our study. Nonetheless, in vitro investigations with larger sample sizes and preclinical studies are needed to confirm the results of this proof-of-concept study and the potential of our radiolabelling approach to be utilised for future in vivo cell tracking studies with the use of PET to allow accurate, non-invasive and sensitive monitoring of stem cell migration and retention to target sites after transplantation.

Abbreviations

^{89}Zr	Zirconium-89
AO/PI	Acridine orange/propidium iodide
CAR T	Chimeric antigen receptor T
CGM	Complete growth medium
Df-Bz-NCS	Para-isothiocyanatobenzyl-desferrioxamine B
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
hPL	Human platelet lysate
HSPC	Hematopoietic stem and progenitor cell
KAERI	Korea Atomic Energy Research Institute
MBq	Megabecquerel
MSC	Mesenchymal stem cell
Necsa	South African Nuclear Energy Corporation
PBS	Phosphate-buffered Saline
PET	Positron emission tomography
SAMRC	South African Medical Research Council

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Author contributions

All authors contributed to the study conception and design. CD, KM, and JM were responsible for the maintenance, expansion and cryopreservation of the MSCs and HSPCs supplied for this study, under guidance of MSP. The import of ^{89}Zr utilised in this study was arranged by JRZ and TE. MK performed all the radiolabelling procedures and experiments. BS, TE, OA and JRZ contributed through research supervision. The first draft of the manuscript was written by MK and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the institutional review board at the Sefako Makgatho Health Sciences University (Ref no. SMUREC/P/21/2017) and informed consent was given to use cryopreserved stem cells from healthy donors.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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