The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	This study employed a controlled experimental design with multiple groups of rabbits subjected to varying concentrations of CNPs to assess staining effectiveness.	Abstract section Line 19
Sample size	2	A total of 48 rabbits were included in the study, The sample size was determined based on previous studies and statistical power analysis to ensure sufficient statistical power.	**Abstract Section** Line 19
Inclusion and exclusion criteria	3	The inclusion criteria for this study are healthy adult rabbits (e.g., New Zealand White) aged approximately 6 months, with a body weight between 2.5 and 3.5 kg, free from diseases or injuries, and ethically approved for use in the experiment. There is no restriction on sex, though the distribution will be balanced between experimental and control groups. The exclusion criteria include rabbits with any illnesses, physical injuries, allergic reactions to carbon nanoparticles, or those in special physiological conditions such as pregnancy or lactation. Additionally, animals outside the specified age or weight range or displaying abnormal behaviors will be excluded from the study.	Method **Section** Line79
Randomisation	4	Animals were randomly assigned to experimental groups based on the concentration of CNPs used for injection.	Method section Line 79
Blinding	5	The investigators performing outcome assessments were blinded to group allocation to avoid bias in data collection.	Method section Line 79
Outcome measures	6	The primary outcome measures will assess the staining efficacy of sentinel lymph nodes, including the intensity and uniformity of CNP uptake. Secondary outcomes will include histological analysis of lymph node tissue for signs of damage, inflammation, or fibrosis, and mRNA expression levels of inflammatory, necrosis, and fibrosis markers. The safety of long-term CNP retention will be evaluated by examining potential toxicity or tissue	Results section Line 129
Statistical methods	7	Data were analyzed using One-Way ANOVA and T-test, and a significance level of $p < 0.05$ was considered statistically significant.	Method section Line 122
Experimental animals	8	The experimental animals will be healthy adult New Zealand White rabbits, 6 months old, weighing 2. 5 – 3.5 kg. They will be free from diseases or injuries and housed under controlled conditions with standard care. Both male and female rabbits will be included, with balanced distribution across experimental and control groups. All procedures will be ethically approved and follow institutional guidelines.	Method section Line 79
Experimental procedures	9	Rabbits will be anesthetized, and carbon nanoparticles (CNPs) will be injected into the subcutaneous tissue near the sentinel lymph nodes at different concentrations. The optimal staining concentration and retention time will be evaluated at 6, 12, and 24 hours. After the retention period, animals will be euthanized, and lymph nodes will be collected for histological analysis and mRNA expression assessment of inflammatory, necrosis, and fibrosis markers .	Method section Line 79
Results	10	The study found that the lowest concentration of CNPs to achieve adequate SLN staining was 0. 0781 mg/0.8 mL, with the best staining results observed at least 12 hours post-injection. Long-term retention of CNPs in the SLNs did not show significant negative impacts on the lymph node structure or function, nor did it alter the mRNA expression levels of inflammatory, necrosis, or fibrosis factors. All raw data are available upon request for transparency and reproducibility of the findings.	Result section Line 129

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	This study investigates the use of carbon nanoparticles (C NPs) for sentinel lymph node staining in rabbit models. The aim is to determine the optimal concentration and retention time for effective staining, while also assessing the biosafety of C NPs. Healthy adult New Zealand White rabbits were injected with various concentrations of C NPs, and staining was evaluated at different time points. Histological analysis, along with mRNA expression of inflammatory, necrosis, and fibrosis mar kers, was conducted to assess tissue response. The results suggest that a concentration of 0.0781 mg/0.8 mL with a 12-hour retention period provides the most effective staining without causing significant tissue damage, offering potential for clinical applications in sentinel lymph node biopsy.	Abstract section
Background	12	Sentinel lymph node biopsy is an essential procedure for the staging of cancers, such as melanoma. The identification of sentinel lymph nodes relies on various methods, including dye injection. However, traditional dyes have limitations in terms of visibility and retention time. Carbon nanoparticles (CNPs) offer an alternative due to their stability and ease of detection. This study aims to optimize CNP staining for sentinel lymph nodes in rabbit models and evaluate its safety profile.	Abstract sec tion Line 20
Objectives	13	The primary objective of this study is to determine the optimal concentration and retention time of carbon nanoparticles (CNPs) for effective sentinel lymph node staining in rabbits. Secondary objectives include assessing the safety of CNPs through histological analysis and the evaluation of inflammatory, necrosis, and fibrosis markers at various time points.	Abstract section Line 20
Ethical statement	14	This study was conducted in accordance with the ethical guidelines for animal research, and all procedures were approved by the institutional animal ethics committee. Efforts were made to minimize animal suffering, and animals were monitored throughout the study to ensure their well-being.	Method sectio n Line 91
Housing and husbandry	15	Rabbits were housed in a controlled environment with a 12-hour light/dark cycle, maintaining temperature between 18 – 22°C and humidity levels between 40 – 60%. They were provided with a standard diet and fresh water ad libitum. Enclosures were large enough to allow free movement, and proper bedding was provided to ensure comfort.	Method section Line 79
Animal care and monitoring	16	The rabbits were monitored closely throughout the study for signs of distress or discomfort. Vital signs, including body temperature, respiration rate, and general health status, were checked daily. Anesthesia was administered during procedures, and appropriate analgesia was provided to manage post-procedural pain. Animals were euthanized at the end of the experiment following approved protocols.	Method sectio n Line 79
Interpretation/ scientific implications	17	The findings of this study provide valuable insights into the potential use of carbon nanoparticles for sentinel lymph node staining. By determining the optimal conditions for CNP injection and retention, this study paves the way for further clinical trials in human patients, potentially improving the accuracy and efficiency of sentinel lymph node biopsy procedures in cancer staging.	Acknowledgeme nts Line 240
Generalisability/ translation	18	While the study was conducted in rabbit models, the findings may be applicable to human clinical settings with further validation. The use of carbon nanoparticles for lymph node staining has the potential to improve the detection and staging of various cancers, contributing to more precise surgical planning and treatment.	Conclusions S ection Line 2 36
Protocol registration	19	The protocol for this study has been registered with IACUC under the registration number 2023-RA-009. The study follows all applicable guidelines for animal research and protocol reporting.	Method sectio n Line 91
Data access	20	Data generated during the study, including histological and molecular analysis results, will be made available upon request. The data will be shared in accordance with institutional policies and ethical guidelines to promote transparency and reproducibility.	Image and Statistics Section Line123
Declaration of interests	21	The authors declare no conflicts of interest related to the research, funding, or outcomes of this study. The study was funded by Medical Guidance Project of the Science and Technology Commission of Shanghai Municipality (22Y11905800) and Center for Scientific Research and Development, Ministry of Education (2021JH014)., and there are no financial relationships that could be perceived as a conflict of interest.	Acknowledgeme nts Line 240