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Nanotechnology - Measurement method for the morphology and size of exosomes - Atomic force microscopy

纳米技术 外泌体形貌和尺寸测量 原子力显微术

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Foreword

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1.Structure and Drafting Rules of Standardization Documents". Drafting. Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed by the Chinese Academy of Sciences. This document is under the jurisdiction of the National Technical Committee on Standardization of Nanotechnology (SAC/TC279). This document was drafted by: National Engineering Research Center for Nanotechnology and Applications, National Center for Nanoscience and Technology, and Guonazhixing (Shanghai) Nanotechnology. Technology Development Co., Ltd., Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Jiangsu Jicui Zhongke Nano Technology Co., Ltd., Suzhou Saifu New Drug Technology Service Co., Ltd., Changzhou Nanjing University High-Tech Research Institute, and Nosai United (Beijing) Biomedical Technology Co., Ltd. Company, Medical Innovation Research Department of the General Hospital of the Chinese People's Liberation Army, Ningbo Sinocare Biotechnology Co., Ltd., Chongqing Qianjiang Wuling Mountain Biomedicine Joint Research Institute, Shandong Shuifa Life Science Research Co., Ltd., Shenzhen Huixin Biomedical Technology Co., Ltd., Shanghai Qirui Biomedical Technology Co., Ltd. Technology Co., Ltd., Shanghai Jiao Tong University, Shanghai Health Medical College, Jinan Fourth People's Hospital Affiliated to

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Based on size and biological origin, extracellular vesicles are mainly classified into three types. apoptotic bodies, microvesicles, and exosomes. Among them, apoptotic bodies... The size is generally from 500 nm to several micrometers, and they originate from cells undergoing programmed cell death; microvesicles are generally 100 nm in size. 1000nm, originating from the budding and separation processes of the cell membrane; exosomes are generally 30nm~150nm in size, originating from the inward migration of cells from the cell membrane. Early endosomes in bud formation are formed from living cells through endocytosis, fusion, and release. Exosomes contain mRNA, miRNA, and non-coding RNA. RNA, DNA, proteins (cytoplasm, cell membrane), lipids, and other complex components are widely present in blood, urine, cerebrospinal fluid, saliva, breast milk, and semen. Peritoneal fluid and synovial fluid, among other biological fluids, participate in intracellular communication and intercellular exchange, and therefore play a crucial role in the study of disease mechanisms. It has high application value in fields such as disease diagnosis and prognosis, drug delivery, immunotherapy, and medical aesthetics. However, it originates from the diversity of cells... The complexity of exosome formation and the differences in separation and purification techniques lead to the high heterogeneity of exosomes, and their unique nanostructures... The high heterogeneity of exosomes makes their characterization and identification a significant challenge. Although the International Extracellular Vesicle Association has proposed the concept of extracellular vesicles... While there are basic requirements and consensus standards for exosome research, there is still no unified standard for the detection and quality control of the physical and biochemical properties of exosomes, both domestically and internationally. One evaluation method. Scanning probe microscopy (AFM) is a technique that uses repulsive forces to obtain surface morphology. AFM can be used for imaging inorganic, polymeric, and biological materials. In this context, imaging of biological materials, especially imaging of membrane particles, requires the following characteristics. 1) In sample pretreatment, the substrate needs to be modified. Biomaterials used for AFM imaging need to be dispersed and

immobilized on a flat substrate, and... Furthermore, the material and the substrate need to have a good bond in order to withstand the erosion and impact of the cleaning solution. 2) During the measurement process, a liquid phase environment should be used as much as possible. In a liquid phase environment, the true morphology and structure of biological materials can be effectively preserved. feature. 3) During the measurement process, non-contact methods should be used as much as possible. Biomaterials are generally soft substances with extremely low elastic modulus and low reaction time to external forces. It should be sensitive and easily damaged by the probe. Based on the above characteristics, this paper utilizes AFM in a liquid phase environment and employs a tapping mode to measure the morphology and size of exosomes. We will establish a consistent method for AFM measurement of exosomes to provide a reference method for AFM measurement of other biological materials. Nanotechnology Exosome Morphology and Size Measurement Atomic force microscopy Warning. This document does not cover all potential security issues. Users are responsible for taking appropriate security precautions before applying this document. Health measures must be implemented and conditions must be met in accordance with relevant national regulations.

1 Scope

GB/T 46972-2026 is the Chinese national standard covering measuring exosomes by AFM - the vesicles cells release to carry signals, thirty to a hundred and fifty nanometres across, soft enough that the tip deforms them, which is the whole difficulty of the measurement. Exosomes are the basis of a large diagnostic and therapeutic industry, and their size distribution is how a preparation is characterised. First edition, under the Chinese Academy of Sciences. It was issued on 28 January 2026 and has been in force since 1 August 2026, as a first edition. The document is under the responsibility of the Chinese Academy of Sciences. This page is published from the official record of the 2026 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

This document describes a method for measuring the morphology and size of exosomes using atomic force microscopy in tapping mode. This document applies to the measurement of exosome morphology and size in a liquid environment; other extracellular vesicles should be measured using the same method.

2 Normative references

The contents of the following documents, through normative references within the text, constitute essential provisions of this document. Dated citations are not included. For references to documents, only the version corresponding to that date applies to this document; for undated references, the latest version (including all amendments) applies. This document.

GB/T 6682-2008 Specifications and test methods for water used in analytical laboratories

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 Extracellular vesicles (EVs) Membrane particles with a lipid bilayer structure released from cells carry and transfer phospholipids and proteins derived from parent cells. Including nucleic acids and other contents, they mediate intercellular signal communication and substance exchange.

3.2 exosome Extracellular structures with membrane structures and generally a size of 30nm to 150nm are produced by "endocytosis, fusion, and release" from living cells. Vesicles.

3.3 By controlling the distance between the probe and the sample surface through detecting the interaction forces (attractive or repulsive forces) between the probe and the sample surface, surface parameters can be obtained. Scanning probe microscopy for morphology. [Source: GB/T 27760-2011, 3.1]

3.4 Tapping mode One operating mode of atomic force microscopy involves driving a microcantilever via a small piezoelectric element mounted within the microscope's probe holder. The probe oscillates near its resonant frequency, causing the probe tip to intermittently contact the sample surface, thus achieving morphological imaging. Note

1.Atomic force microscopes have different operating modes, namely contact mode, non-contact mode and tapping mode. Note

2.In contact mode, the needle tip is always in contact with the sample, and there is a repulsive force between the needle tip and the sample, which is suitable for highly elastic models or samples that are not easily deformed. Note

3.In non-contact mode, the needle tip never contacts the sample. The interaction between the needle tip and the sample is a long-range force, and the resolution decreases when the distance between the needle tip and the sample is relatively long.