The rise of nanomechanical biomarkers: how cell and tissue mechanics can help medicine

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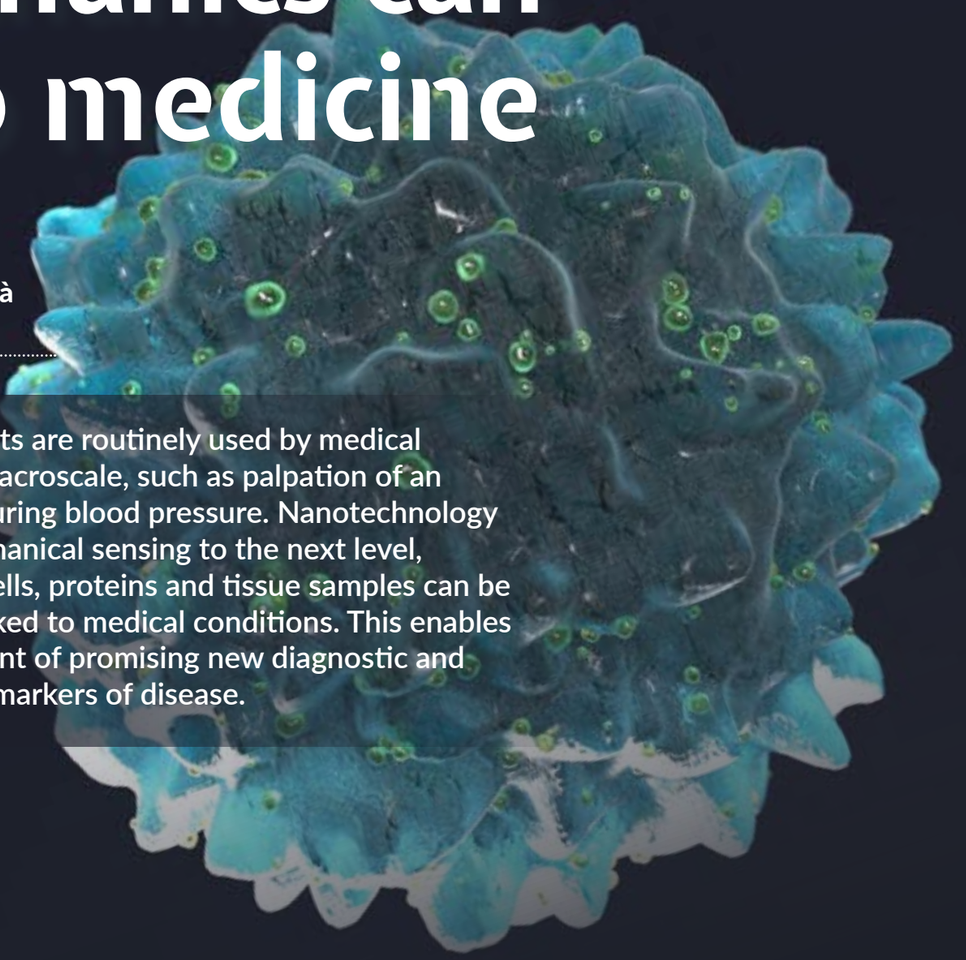
Mechanical tests are routinely used by medical doctors on a macroscale, such as palpation of an organ or measuring blood pressure. Nanotechnology has taken mechanical sensing to the next level, where single cells, proteins and tissue samples can be probed and linked to medical conditions. This enables the development of promising new diagnostic and prognostic biomarkers of disease.



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MechaDynA

What is a biomarker?

In 1500 BC, Hindu scholars observed that the urine of diabetic patients attracted ants and flies (Das, 2011). We now know that this happens because of its high sugar content. Still, it was probably the first reported signature, or biomarker, of a physiological state or medical condition. The European Medicines Agency (EMA) defines a biomarker as an objective and quantifiable measure of a physiological process, a pathological process or a response to treatment (excluding measures of how an individual feels or functions). Thus, biomarkers can be used to diagnose or prognose disease or assess a treatment's success. Most biomarkers are molecular, relying on the detection of specific molecules. For example, antigen tests for COVID-19 detect specific molecules in our body fluids to assess the presence of the SARS-CoV-2 virus. In this case, we would need to know which molecule to target. Biomarkers are also widely used in cancer diagnosis: the presence (or absence) of certain molecular markers in biopsy samples allows us to identify the type of tumour or its prognosis.

However, not all biomarkers are based on molecules. For example, body temperature or the number of white blood cells in our blood are established biomarkers related to inflammatory conditions. More recently, electrocardiograms or records of the heart's electrical activity are one of the most used biomarkers of heart disease. In an even more direct way, just as we check whether a piece of fruit is ripe, doctors palpate organs from patients to look for possible markers of disease.

Mechanical properties of cells and tissues can be used as biomarkers

Physical examination through palpation of the body was already reported by Hippocrates (460–370 BC) and is a long-standing practice in medicine to detect a medical state based on mechanical properties, such as stiffness (Das and Shah, 2011). The observation of alterations in the rigidity of organs and tissues is still currently used to detect and diagnose certain diseases, for example, as a first approach for early detection and localisation of tumours. Tactile exploration of the abdominal cavity to feel the tenderness of the intestines can help diagnose appendicitis. Palpation is also used to assess the pathophysiological state of organs, like the liver, and it is an essential technique in physiotherapy to assess the state of the patient's bones, joints and muscles. The elasticity of our skin is also an obvious example of a mechanical biomarker that may reveal the biological age of individuals: in general, skin from young individuals is very elastic while, in contrast, aged skin often shows wrinkles due to changes in mechanical properties and is looser and more flaccid. Another widely used mechanical property in medicine is blood pressure, which can be associated with cardiovascular diseases. Therefore, the mechanical properties of certain tissues and organs allow discerning normal from pathological conditions.

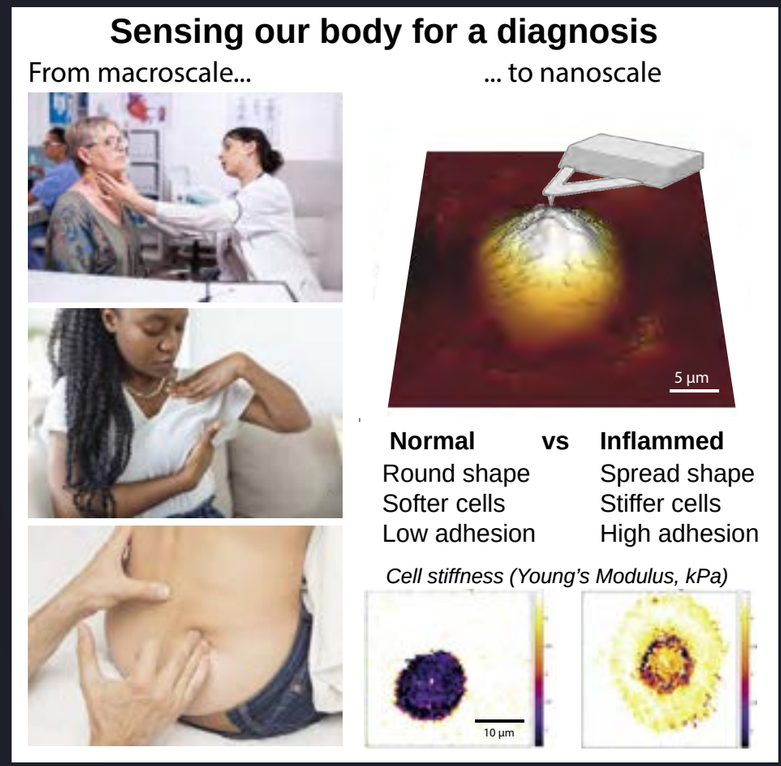

Figure 1: Mechanical properties are used by medical doctors at the macroscale to diagnose disease through palpation tests (Images by DCStudio on Freepik).

Atomic force microscopy (AFM) uses a flexible cantilever to measure mechanics at the nanoscale, as depicted on this 3D view of a white blood cell. This can also give pathophysiological insight (cantilever image from Biorender). For example, certain white blood cells appear soft in normal conditions (bottom left) but stiffen and become more adherent when activated during inflammation (bottom right). Brighter colours represent higher stiffness values (Eroles et al., 2023).

Nanotechnologies allow mechanical exploration at the smallest scales

The development of nanotechnologies since the 1980s has opened the door to the mechanical characterisation of molecules, cells and tissue slices, at the micro- and nanometre scale, i.e. down to 1 000 000 times smaller than a millimetre.

For example, atomic force microscopy (AFM) uses a tiny probe to touch the sample, apply a controlled force or pressure and quantify the system's

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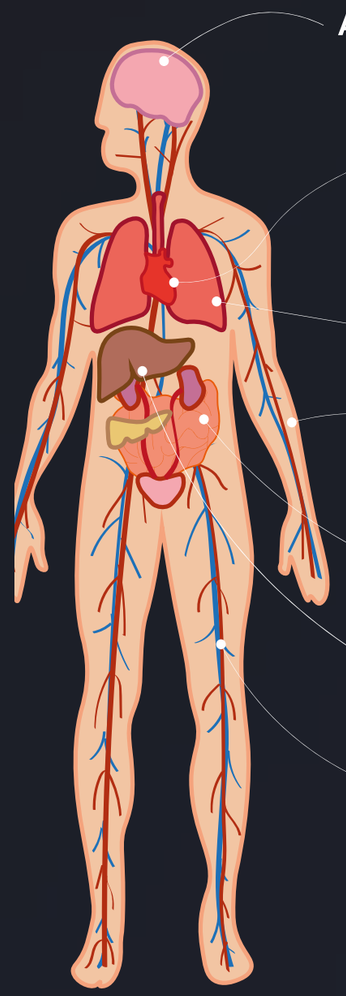

Ageing
Alzheimer's disease, *e.g. brain tissue softening*
Osteoporosis, *e.g. bone with lower fracture resistance*
Skin wrinkling, *e.g. skin softening*

Cardiovascular diseases
Hypertension, *e.g. arterial stiffening*
Heart failure, *e.g. arterial stiffening*
Hypertrophic cardiomyopathy, *e.g. cardiac tissue stiffening*
Congenital hypertrophies, *e.g. cardiac tissue stiffening*

Respiratory diseases
Pulmonary fibrosis, *e.g. microenvironment stiffening*
SARS-CoV-2 infection (COVID-19), *e.g. leukocytes softening*

Cancer
Breast, lung, ovarian, bladder, pancreatic, colorectal, prostate cancers and leukemia,
e.g. cell softening, microenviroment stiffening

Autoimmune diseases
Inflammatory bowel disease, *e.g. extracellular matrix stiffening*
Systemic lupus erythematosus, *e.g. red blood cells stiffening*

Inflammatory diseases
Sepsis, *e.g. arterial stiffening*
Hepatitis, *e.g. liver stiffening*

Hematopathies
Sickle cell disease, *e.g. red blood cells stiffening*
Malaria infection, *e.g. red blood cells stiffening*

Figure 2: Nanomechanical biomarkers in biomedicine. The mechanical properties of cells and tissues are affected in a wide range of human diseases, from immune disorders to cancer age-related conditions. Their quantification in patients has the potential to improve personalised medicine and the study of human pathologies.

stiffness, as shown in Figure 1. Mechanical properties of cells can also be measured using microfluidics and advanced computation tools, such as deformation cytometry. Using these techniques, mechanical changes in biological samples can be detected with high accuracy, robustness, and reproducibility (Perez-Dominguez *et al*,. 2023).

Nanomechanical biomarkers

Nanotechnology allowed the flourishment of multiple studies in biomedicine from a nanomechanical perspective. In this context, cancer has attracted a lot of attention. Cancer cells are softer than normal cells, whereas the extracellular matrix surrounding the tumour tends to be stiffer and more rigid (Lekka *et al*., 1999; Plodinec *et al*., 2012). This affects the aggressiveness of the tumour and the effectiveness of specific treatments. Clinical trials have started to assess the possibility of using the mechanical signature of biopsies from cancer patients to evaluate the tumour condition, malignancy and response to treatment (ARTIDIS AG, 2023).

Cancer is not the only disease that may present a nanomechanical signature or biomarker. As shown by Figure 2, nanomechanical biomarkers can also be used in cardiovascular diseases, respiratory diseases, hepatopathies, inflammatory diseases and autoimmune diseases. During the COVID-19 pandemic, the stiffness of blood cells was measured, showing that certain white blood cells were softer in COVID-19 patients than in healthy or recovered individuals (Kubánková *et al*., 2021).

Advantages of nanomechanical biomarkers

From these few examples, we can see a clear rise of nanomechanical biomarkers in health and disease. Nanomechanical biomarkers provide important advantages compared to conventional ones (Eroles and Rico, 2023). First, working at the nanoscale allows the use of very small quantities of biomaterial (blood samples, biopsies). Second, nanomechanical biomarkers do not require specific labelling or molecular synthesis, as in antigen tests, for instance, allowing faster development. Third, mechanical properties are very sensitive to physiopathological conditions, allowing us to detect earlier changes that may not be immediately reflected by other markers, such as protein expression. Finally, nanomechanics take advantage of precise and robust nanotools and are suitable to provide highly quantitative biomarkers.

Current challenges and future perspectives

The field of biomarkers is constantly changing with advances that provide faster and more accurate medical tools. As a result, mechanical biomarkers are gaining applications and being designed to perform better. Companies like ARTIDIS or Rivercyte are emerging and are helping to establish mechanical biomarkers to diagnose diseases. However, each new niche faces challenges in gaining market share. Nanotechnology is evolving towards more accessible and automatised tools that are easier to use and that provide higher throughput, bringing nanomechanical biomarkers closer to the medical practice. Standardisation of techniques, good practices and homogenised data acquisition and processing across techniques are pivoting in this growing field of translational research. In addition, broadening the horizons of scientists, medical doctors and companies will be key to the acceptance of nanomechanical biomarkers in hospitals and clinics. Finally, outreach is also necessary to allow a dialogue between researchers and society, listen to its needs and raise awareness and demand of new technologies. To achieve this, EU research programmes, such as the ERC grants and MSCA collaborative networks, concentrate on these key players and help bring nanomechanical biomarkers closer to patients.

The ERC project MechaDynA develops and applies nanotools to explore the limits of the mechanical response of immune cells in search of fundamental physics principles. This has led to the development of approaches that accelerate and automate the acquisition and analysis of mechanical measurements, with potential future applications in the clinic. For instance, how white blood cells modulate their adhesion and mechanical properties upon reaching the inflamed tissue is still not well understood. We used various nanotools to quantify the morphology, adhesion, and viscoelasticity of monocytes and differentiated macrophages (Eroles *et al*., 2023) . The H2020-MSCA-ITN project Phy2BioMed assembled academic partners, hospitals and nanotechnology companies to train a new generation of early-stage researchers as experts in cell and tissue nanomechanics. Phys2BioMed explored different applications of nanomechanics in health and disease, implemented and compared different techniques, and developed standardised approaches and data analysis tools (Gerum *et al*., 2022; Lekka *et al*., 2023; Lopez-Alonso *et al*., 2023; Pérez-Domínguez *et al*., 2023). All these advances are based on optimisation efforts and also on a better understanding of fundamental questions. Citing Pasteur: "There is no such thing as applied research, only research applications." Through fundamental research, innovation and dialogue between disciplines, nanomechanical biomarkers are now closer to becoming a clinical tool.

References click here

PROJECT NAME
MechaDynA

PROJECT SUMMARY
"MechaDynA" develops two novel nanotools to allow force measurements on living cells. The goal is to obtain a complete, multi-scale picture of the physics of leucocyte adhesion. Technologically, it will establish two nanotools for force measurements on living cells covering the widest temporal range. This will open the door to unexplored physical phenomena in cell biology, biological physics and soft condensed matter. Biomedically, the expected outcomes will provide a mechanistic description of the physics of leucocyte immune response that may lead to better diagnosis and therapeutics.

"Phys2BioMed" aims to identify biomechanical markers for the early detection of disease. The objective is to create an interdisciplinary research training network for early-stage researchers on the application of physical tools for the mechanical phenotyping of cells and tissues applied to early diagnostics. The network merged training from fields such as nanotechnology, physics, biology and medicine, defining the main features of new-generation instrumentation optimised for the mechanical phenotyping of clinical samples.

PROJECT LEAD PROFILES
Felix Rico is *Maître de Conférences* (associate professor) at the Physics Department of Aix-Marseille University (AMU), Claire Valotteau is *Chargée de recherche* (permanent researcher) at the CNRS. **Mar Eroles** is a postdoctoral researcher at DyNaMo. Their research is being developed at DyNaMo, a joint Inserm and AMU laboratory. They develop and apply force microscopy tools to unravel the physics behind biological systems.

Alessandro Podestà is associate professor at the Physics Department of Università degli Studi di Milano. He studies nanostructured systems and interfaces at the nanometre scale.

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