Role of nanoparticle mechanical properties in targeted cancer drug delivery

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VOCABULARY:

Mechanical properties - physical properties that a material exhibits upon the application of forces. **Indentation** - a common method of testing the mechanical properties of materials by pressing the materials using an indenter tip. **Force-displacement curve** - a curve specifying the displacement of an object that results from applied forces. **Biodistribution** - a method of tracking where nanoparticles of interest

travel in an experimental animal or human subject. **Endocytosis** - the cellular process in which substances are surrounded by plasma membrane and brought into the cell.

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ABSTRACT

The physicochemical properties of nanoparticles play critical roles in regulating nano-bio interactions. While the effects of the size, shape and surface charge of nanoparticles on their biological performances have been extensively investigated, roles of nanoparticle mechanical properties in drug delivery which has only been recognized recently remains least explored. This review article provides an overview of the impacts of nanoparticle mechanical properties on targeted drug delivery, including (1) basic terminologies of the mechanical properties of nanoparticles and techniques for characterizing these properties; (2) current methods for fabricating nanoparticles with tunable mechanical properties; (3) in vitro and in vivo studies that highlight key biological performances of stiff and soft nanoparticles, including blood circulation, tumor or tissues targeting, tumor penetration, and cancer cell internalization, with a special emphasis on the underlying mechanisms that control those complicated nano-bio interactions at the cellular, tissue and organ levels. The interesting research and new findings discussed in this review article will offer the research community a better understanding of how this research field evolved during the past years, and provide some general guidance on how to design and explore the effects of nanoparticle mechanical property on nano-bio interactions. These fundamental understandings will in turn improve our ability to design better nanoparticles for enhanced drug delivery.

Advances in nanotechnology have led to rapid development of the synthesis, characterization and applications of nanoparticles (NPs) in cancer treatment. Various NP-based drug delivery systems have been designed to deliver therapeutic agents specifically to solid tumors to enhance the efficacy of anticancer treatment while minimizing systemic toxicity.¹ The enhanced permeability and retention (EPR) effect has long been considered as the key mechanism to facilitate the preferential accumulation of NPs in tumor tissues compared to normal tissues.^{2,3} However, recent studies have raised questions about the actual benefits of this biological phenomenon. The systemic transport of NPs to solid tumors following intravenous (*i.v.*) administration is never trivial, but extremely complicated involving several key biological barriers at all levels from organs and tissues to cells. In general, NPs encounter five key biological barriers from the injection site to the site of action, including (1) adsorption of serum opsonin proteins onto the surface of NPs,⁴ (2) interactions between NPs and the immune system,⁵ (3) selective extravasation of NPs at tumor sites,² (4) penetration of NPs into solid tumor, and (5) internalization of NPs by tumour cells. The eradication of tumor cells requires NPs to overcome the dense tumor extracellular matrix and high interstitial fluid pressure in the tumor tissue,⁶ as well as to efficiently enter cancer cells.⁷ Due to the overwhelming complexity of these processes, targeted cancer drug delivery often exhibit suboptimal therapeutic benefits, with only 0.7% (median) of the administered NPs reported to reach tumor sites.^{1,8}

NP size,⁹⁻¹¹ shape,¹²⁻¹⁴ surface chemistry and other physicochemical properties^{14,15} have been extensively studied, and their effects on cancer targeted drug delivery have been systematically investigated. However, the mechanical property of NPs has only recently been recognized to play an important role in regulating their biological performances. This is largely inspired by the facts that many cells or even viruses can modulate their

mechanical properties to achieve certain biological functions.^{16,17} Despite the growing evidences highlighting its pivotal role in biology, a consensus about the correlation between the mechanical property of NPs and their biological fates is still lacking, as manifested by the conflicting views among different studies. This review focuses on the role of NP mechanical property in targeted drug delivery. Basic terminologies of NP mechanical property including stiffness and elasticity are defined. The commonly used methods for quantifying NP mechanical property are described, followed by discussion on the approaches for making NPs with tunable mechanical property but identical size, shape and surface property. Furthermore, the effects of NP mechanical property on several key biological processes involved in systemic drug delivery are highlighted, including blood circulation, tumor accumulation/penetration, and cancer cell internalization, with a particular emphasis on the mechanisms underlying the distinct biological performances of soft and stiff NPs using experimental and computational approaches. As we focus on the mechanical property of NPs and its role in regulating their biological performance, studies on micron-sized particles with tunable mechanical property¹⁸⁻²⁴ will not be discussed here.

BASIC TERMS AND DEFINATIONS OF MECHANICAL PROPERTIES

Mechanical properties describe the behavior of a material under a load. Several key properties include elasticity, stiffness, rigidity, hardness, and strength, which have different definitions and different units. Among these mechanical terms, elasticity and stiffness have been widely used in literature exploring the mechanical properties of NPs (**Table 1**). In physics, elasticity is defined as the ability of a material to resist deformation induced by stress, and to return to its original state when that stress is

removed. The elasticity of a material is normally presented as its elastic moduli (N/m^2 or Pa), including Young's modulus and shear modulus for linear elastic isotropic materials (**Figure 1a**). Young's modulus (E_Y) is defined as the ratio between the uniaxial tensile/compressive stress applied on a material and its corresponding strain along the direction of the stress. Shear modulus (G), on the other hand, is the ratio of shear stress to the corresponding shear strain of a material. Another essential mechanical property term, stiffness (N/m), is often used interchangeably with rigidity ($\text{N}\cdot\text{m}$) to characterize the extent to which a body resists deformation upon the application of loadings. Unlike elastic moduli, which are intrinsic factors determined only by the material itself, the stiffness of an object is extensive which not only related to its elastic moduli, but also its dimension, shape and the directionality of applied forces. In general, the stiffness of an object can be described using Hooke's law, in which the stiffness (also called spring constant) of a spring (**Figure 1b**) is a ratio of the compressive/tensile force applied on it to its compression/extension. Similarly, the compressive/tensile stiffness of an object measures its ability to resist deformation induced by compressive and tensile forces, while the bending stiffness and torsional stiffness ($\text{N}\cdot\text{m}/\text{rad}$) are the abilities to resist bending or torsional deformation caused by bending forces or torques (**Figure 1b**). It should be mentioned that even for the same object, its compressive/tensile stiffness, bending stiffness and torsional stiffness can be very distinct. For example, it is normally easier to bend a beam than to lengthen it. Because the most common type of force applied on NPs is compression, Young's modulus and compressive stiffness (simply referred to stiffness) are the most relevant characteristics within the context of this review article. However, since the stiffness of NPs is also affected by their structures and geometrics, using Young's modulus allows a more straightforward comparison between the mechanical properties of different

materials. In this review, the effects of NP mechanical property on biological performances will be summarized based on Young’s modulus (elasticity).

Table 1. The elasticity and stiffness of a nanoparticle.

Mechanical property	Definition	Expression	Unit	Feature
Elasticity	The ability of an object to resist deformation caused by stress and to return to original state when the stress is removed.	Young’s modulus: The ratio between the uniaxial tensile/compressive stress and strain.	Pascal (Pa or N/m ²)	<ul style="list-style-type: none">• An intrinsic property of the material;• Independent of geometry;• Elastic limit exists^a.
Stiffness	The extent to which a body resist deformation caused by applied forces.	The ratio between applied force and the corresponding deformation.	Newton per meter (N/m)	<ul style="list-style-type: none">• Determined jointly by the elastic modulus and geometry of an object;• Readily derived from the indentation force–displacement curve.

^a Elastic limit is the maximum stress that can arise in a material before the onset of permanent deformation.

Figure 1. Schematic illustrations of basic mechanical property terms and their definitions. (a) Elasticity and elastic moduli. (b) Spring constant and stiffness. (c) Membrane bending rigidity.

Atomic force microscopy (AFM) has been the most popular technique for characterizing the mechanical properties of NPs. AFM uses a nano-sized probe to scan

a sample surface and determine its surface topology. To measure the overall mechanical property of a NP, a typical way is to exert a compressive force on the NP to cause deformation. Then, both the applied force and the deformation of the NP are recorded in the form of an indentation force–displacement curve. According to Hooke’s law, the stiffness of a sample is equal to the slope of the linear part of the curve, whereas the calculation of Young’s modulus involves fitting the force–displacement curve using certain contact models, such as the Hertz model and Sneddon model.²⁵⁻²⁹ The classical Hertz model, which is only valid at the indentation depth much smaller than the indenter tip radius, is generally not applicable for soft materials such as biological tissues and liposomes. To achieve a more appropriate estimation of the Young’s modulus of soft materials undergoing finite deformation, the Sneddon theory is usually adopted.²⁹ This indentation curve method is only able to provide one data point for a single indentation experiment, and is very time-consuming to obtain high-resolution Young’s modulus maps. A PeakForce Quantitative Nanomechanical Mapping (QNM) tapping mode AFM has been developed recently,^{30,31} which allows the acquisition of mechanical information of samples while simultaneously imaging the topography at a high resolution and can operate as fast as the regular tapping-mode AFM.³¹ Due to its high performance and easy operation, the PeakForce QNM mode has been used in several studies to measure the mechanical properties of NPs.³²⁻³⁴ Another method for quantifying NP mechanical property is to measure bulk materials at the macroscopic scale rather than at the nanometer scale.³⁵⁻³⁷ This approach has only been applied to hydrogel NPs because bulk hydrogel materials can be easily synthesized to have identical chemical composition, thus the same Young’s modulus as the corresponding hydrogel NPs. Measurement of the mechanical properties of bulk hydrogel materials can be conducted by applying macroscopic forces (e.g., using rheometer³⁷) on

specimens and analyzing their force–deformation profiles. It is worth noting that Young’s modulus is a parameter of homogenous and isotropic materials such as the aforementioned hydrogel materials. Therefore, the measured Young’s moduli of composite NPs (e.g., core–shell NPs) are actually more of effective moduli considering the NPs as uniform materials.

Besides the mechanical properties of NPs, the bending rigidity of biological membranes also plays a critical role in dictating how cells interact with NPs of different mechanical properties. Membrane bending rigidity κ (N·m) measures the mechanical resistance of a membrane to loadings (normally bending moments) (**Figure 1c**). It has become an important parameter in computational simulations to mechanistically understand the membrane deformation and wrapping of stiff and soft NPs.^{38–40} Methods for measuring the bending rigidity of biological membranes include fluctuation spectroscopy⁴¹ and micropipette aspiration.⁴²

APPROACHES FOR SYNTHESIZING NANOPARTICLES WITH CONTROLLABLE MECHANICAL PROPERTIES

To investigate the effects of NP mechanical properties on targeted cancer drug delivery, a key prerequisite is the synthesis of NPs having distinct mechanical properties but identical in other physicochemical properties (e.g., size, shape and surface chemistry). In this section, current approaches for synthesizing NPs with tunable mechanical properties are introduced, covering hydrogel NPs, hybrid polymer–lipid NPs and silica nanocapsules that have been recently examined in depth towards the fundamental understanding of the impacts of NP mechanical property on drug delivery applications (**Table 2**).

Table 2. Representative examples of nanoparticles with varying mechanical property and the effects of mechanical property on their biological performances.

Materials	Size (nm)	Modulus	Effects on biological performances	Ref
Hydrogel nanoparticles (NPs)				
N,N-diethyl acrylamide; 2-hydroxyethyl methacrylate	~160	18-211 kPa	<ul style="list-style-type: none">Soft and stiff NPs are internalized by RAW264.7 macrophage cells mainly through macropinocytosis and clathrin-mediated routes, respectively;NPs with medium elasticity are internalized <i>via</i> multiple mechanisms, which leads to a higher cellular uptake rate compared to soft and stiff NPs.	43
Poly(ethylene glycol) diacrylate	~210	10-3000 kPa	<ul style="list-style-type: none">Soft NPs display longer <i>in vivo</i> blood circulation and enhanced tissue targeting compared to stiff NPs;Soft NPs exhibit lower <i>in vitro</i> uptake in immune cells (J774 macrophage), endothelial cells (bEnd.3) and cancer cells (4T1) compared to stiff NPs.	37
Poly(2-hydroxyethyl methacrylate)	~1000	15-156 kPa	<ul style="list-style-type: none">Soft particles show faster and higher uptake by HepG2 cells than stiff particles.	35
Poly(carboxybetaine)	~120	180-1350 kPa	<ul style="list-style-type: none">Softer NPs show longer blood circulation half-lives than their stiffer counterparts;Stiffer NPs display higher splenic accumulation than softer NPs.	36
Methoxypoly(ethylene glycol)-poly(lactide)	Small: 17 (soft) 18 (stiff) Medium: 34 (soft) 51 (stiff) Large: 68 (soft) 86 (stiff)	Soft: 165 kPa Stiff: 260 kPa	<ul style="list-style-type: none">Stiff NPs display enhanced melanoma A375 cellular uptake and tumor spheroid penetration compared to soft NPs;The elasticity-dependent uptake effect is more significant at larger NP sizes, indicating a coupling effect between NP stiffness and size in regulating cellular uptake.	44
Hybrid polymer–lipid NPs				
PLGA ^a –Lipid monolayer PLGA ^a –Lipid bilayer	~100	No quantitative data. The lipid-bilayer NP is stiffer than the lipid-monolayer NP.	<ul style="list-style-type: none">Stiff NPs exhibit greater <i>in vitro</i> cellular uptake and more significant <i>in vivo</i> anticancer effect than soft NPs.	34

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Alginate–Lipid bilayer	~160	45-19000 kPa	<ul style="list-style-type: none">Soft NPs exhibit higher cellular uptake than stiff NPs by both neoplastic and non-neoplastic cells;Soft NPs accumulate more than stiff NPs in the orthotopic breast tumor model <i>in vivo</i>.	45
PLGA ^a –Lipid bilayer	~200	5-110 MPa	<ul style="list-style-type: none">Semi-elastic NPs (50 MPa) exhibit superior mucosal and tumor penetrating capability compared to soft and stiff NPs;Orally administered semi-elastic NPs efficiently overcome multiple intestinal barriers, enhancing the bioavailability of Dox.	32
PLGA ^a –Lipid bilayer	~40	Soft: 0.76 GPa	<ul style="list-style-type: none">Stiff NPs display higher uptake than the soft NPs by HeLa cells;	33
PLGA ^a –Water–Lipid bilayer		Stiff: 1.20 GPa	<ul style="list-style-type: none">Both soft and stiff NPs are internalized through clathrin-mediated endocytosis.	
Organosilica nanocapsules (SNCs)				
Thioether-bridged silica NPs or NCs ^b	~290	Stiff: 233 MPa Soft: 48 MPa	<ul style="list-style-type: none">Soft SNCs show a 26-fold higher uptake by MCF-7 cells than stiff silica NPs;Soft drug-loaded SNCs display more significant killing effects for MCF-7 cells than the stiff silica NPs.	46
Benzene-bridged silica NPs or NCs ^b	~235	Stiff: 351 MPa Soft: 91 MPa		
Ethane-bridged silica NPs or NCs ^b	~280	Stiff: 251 MPa Soft: 4 MPa		
SNCs with varying shell thickness, prepared using tetraethyl orthosilicate or triethoxyvinylsilane	~200	704 kPa-9.7 GPa	<ul style="list-style-type: none">Either naked or PEGylated stiff SNCs show higher macrophage uptake than their soft counterparts;PEGylated stiff and soft SNCs exhibit similar cancer cellular uptake;Folic acid-PEG-modified stiff SNCs show significantly higher receptor-mediated cellular uptake than the soft SNCs;Soft SNCs show superior tumor penetrating ability than the stiff SNCs;Stiff SNCs display higher splenic removal <i>in vivo</i>, therefore inferior tumor targeting efficiency than soft SNCs.	47

^aPoly(lactic-co-glycolic acid);

^bSilica nanocapsules selectively etched, hollow silica nanoparticles.

Hydrogel nanoparticles

A hydrogel is a cross-linked network of hydrophilic polymeric chains surrounded by a water-rich environment.⁴⁸ Hydrogels can be made into colloidal NPs and their mechanical properties can be tuned by adjusting the degree of crosslinking, hence water content, through changing the amount of crosslinking agents during the hydrogel NP formation (**Figure 2a** left). For example, by increasing the crosslinker content from 1.7 mol% to 15 mol%, N,N-diethyl acrylamide and 2-hydroxyethyl methacrylate hydrogel NPs were fabricated having Young's moduli ranging from 18 kPa to 211 kPa but similar sizes and zeta potentials.⁴³ An alternative way to tune the mechanical properties of hydrogel NPs is by controlling the concentration of monomers during synthesis (**Figure 2a** right). Polyethylene glycol diacrylate hydrogel NPs were synthesized having elastic moduli of 10 kPa and 3000 kPa using 10 vol% and 40 vol% of monomers, respectively.³⁷ Other than the above two methods, a lyophilization method has also been developed to tune the elasticity of hydrogel NPs. Polymer micelles formed by the self-assembly of di-block co-polymers of poly(ethylene glycol)-poly(lactide) (PEG-PLA) in aqueous solutions exhibited lower Young's modulus (165 kPa) owing to the higher water content, whereas an additional lyophilization step dehydrated the micelle, resulting in an increased Young's modulus (260 kPa).⁴⁴ More studies describing the fabrication of hydrogel NPs with tunable mechanical properties are listed in **Table 2**.

Figure 2. Strategies for tuning the mechanical property of nanoparticles. (a) Controlling the mechanical property of hydrogel nanoparticles by changing the concentration of crosslinking agent (left) or monomer (right), thus the crosslinking density of the nanoparticle. (b) Controlling the mechanical property of hybrid polymer–lipid nanoparticles by changing the crosslinking degree of the inner polymeric core (left) or by altering the thickness of the water layer (right) between the polymeric core and the lipid shell. (c) Controlling the mechanical property of silica nanocapsules by using different types of silica precursors (organosilane for soft nanocapsules and silicon

alkoxide for stiff nanocapsules) (left) or by varying the thickness of the silica shell (right).

It should be pointed out that despite the easy control over their mechanical properties, hydrogel NPs only exhibit Young’s moduli ranging from dozens of kPa to a few MPa due to their intrinsic water-rich structures (**Figure 3**). Therefore, even at their ‘stiff’ state, hydrogel NPs are softer than other mechanically stiff materials such as polymeric and silica NPs.

Figure 3. A spectrum of the mechanical properties of different nanoparticles. Hydrogel nanoparticles normally have Young’s moduli ranging from dozens of kPa to a few MPa. The Young’s moduli of polymer–lipid nanoparticles can be down to kPa or up to GPa ranges. However, for a certain core material, it remains challenging to adjust its mechanical property over a broad range. Silica nanocapsules display a very broad mechanical property range, with Young’s moduli ranging from kPa to GPa. Refer to

Table 2 for the biological effects due to the varied mechanical properties of these nanoparticles.

Hybrid polymer–lipid nanoparticles

Hybrid polymer–lipid NPs represent a family of materials having a core–shell structure. In general, polymer–lipid NPs are composed of a polymeric NP core and a lipid monolayer or bilayer as the shell, and their mechanical properties can normally be tuned in two ways. One is to change the crosslinking extent of the inner polymeric core (**Figure 2b** left) while the other is to vary the amount of water between the polymeric core and the lipid shell (**Figure 2b** right). By controlling the crosslinking degree of alginate hydrogel NPs covered by a lipid bilayer, polymer–lipid NPs having Young's moduli ranging from 1.6 MPa to 19 MPa were made.⁴⁵ Alternatively, increasing the water content between the polymeric core and the lipid shell decreases the Young's moduli of the NPs. Two types of hybrid NPs with different amount of water were prepared using a microfluidic device, that is, poly(lactic-co-glycolic acid) (PLGA)–lipid NPs (less water content) and PLGA–water–lipid NPs (more water content), which displayed Young's moduli of 1.20 GPa and 0.76 GPa, respectively.³³ The same strategy was also adopted³² to synthesize PLGA–water–lipid NPs with variable mechanical properties. As their water content decreased, the Young's moduli of the NPs increased from 5 MPa to 110 MPa.

Compared to the lipid shell, the polymeric core mainly contributes to the overall mechanical property of a hybrid polymer–lipid NP. For example, by excluding the polymeric core from the hybrid NPs to form hollow lipid shells (liposomes), the Young's modulus decreased significantly from 19 MPa down to 45 kPa.⁴⁵ Polymer–

lipid NPs can be very soft with Young's moduli as low as 1.6 MPa when hydrogel NPs are encapsulated,⁴⁵ while they can also be very stiff (e.g., 1.2 GPa) when containing dense polymeric NPs.³³ However, it remains challenging to produce polymer–lipid NPs with identical size, shape and surface chemistry, but varied mechanical properties covering a wide range of Young's modulus from kPa to GPa (**Figure 3**).

Silica nanocapsules

Silica nanocapsules (SNCs) have recently emerged as an attractive nanoparticle system having controllable mechanical property. A SNC is a nano-sized core–shell structure with a core region surrounded by a silica shell.⁴⁹ When silicon alkoxides (e.g., tetraethyl orthosilicate (TEOS) and tetramethyl orthosilicate (TMOS)) are used as silica precursors, the resultant silica shells are very rigid. However, by using organosilanes containing organic groups (e.g., vinyl, thioether, benzene, ethane, and methyl), soft and deformable silica shells can be prepared as a result of the low crosslinking density (**Figure 2c** left).⁵⁰⁻⁵²

Thioether-bridged, benzene-bridged and ethane-bridged mesoporous organosilica nanocapsules were synthesized using bis[3-(triethoxysilyl)propyl] tetrasulfide, 1,4-bis(triethoxysilyl)benzene and 1,2-bis(triethoxysilyl)ethane as silica precursors respectively, in combination with a preferential etching approach.⁴⁶ These etched SNCs displayed good deformability and much lower Young's moduli (48 MPa, 91 MPa and 3 MPa, respectively) compared to the non-etched SNCs prepared using the same precursors (233 MPa, 351 MPa and 251 MPa, respectively). The mechanical property of SNCs can also be tuned by varying the silica shell thickness (**Figure 2c** right). We recently reported the fabrication of nanoemulsion-templated stiff and soft SNCs by

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3 using TEOS and an organosilane, triethoxyvinylsilane (TEVS), as precursors.⁴⁷ By
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5 increasing the reaction time from 30 h to 50 h, the silica shell thickness can be increased
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7 from ~10 nm to ~25 nm, resulting in an increase in stiffness from 0.04 N/m to 0.4 N/m
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9 for the soft TEVS SNCs and from 8.7 N/m to 20.1 N/m for the stiff TEOS SNCs. The
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11 derived Young's moduli of the softest and the stiffest SNCs were 704 kPa and 9.7 GPa,
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13 respectively.
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18 It is worth noting that due to the different silica precursors used for making soft and
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20 stiff SNCs, different surface groups can be introduced onto the surfaces of the SNCs.⁴⁹
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22 To take advantage of the facile functionalization of the silica shells, surface
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24 modification such as PEGylation, is normally adopted to render the surface of soft and
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26 stiff SNCs identical and functional.^{46,47} PEGylation does not significantly change the
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28 mechanical property of SNCs as it is the silica shell (thickness and chemistry) that
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30 mainly determines the Young's modulus of SNCs.⁴⁷ As reflected in the above examples,
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32 the mechanical property of SNCs can be adjusted in a very broad range, from kPa to
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34 GPa (**Figure 3**), which makes it possible to systematically study the effect of NP
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36 mechanical property on nano-bio interactions.
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45 EFFECTS OF NANOPARTICLE MECHANICAL PROPERTY ON 46 47 TARGETED CANCER DRUG DELIVERY 48 49

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51 Generally, NP-based drug delivery systems administered *via* intravenous injection go
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53 through a series of biological processes from the injection site to the side of action,
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55 including the formation of protein corona, the clearance by immune system, the
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57 selective accumulation and penetration in solid tumors, and the internalization by
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59 cancer cells. This section reviews latest studies on the crucial roles of the mechanical
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properties of NPs in their blood circulation, tumor targeting and biodistribution, tumor penetration and tumor cell internalization (**Table 2**).

Blood circulation

The blood circulation time of NPs has long been recognized to be positively correlated with their accumulation in the tumor tissue.⁵³ Therefore, great efforts have been attributed to design NPs to have prolonged circulation time by overcoming biological barriers, including immune cell uptake and blood filter. This is commonly achieved by rationally engineering the physicochemical properties of NPs such as size, shape and surface charge.^{53,54} Recently, the mechanical property of NPs has also proven to be an important parameter that can be leveraged to regulate their blood circulation time.

The circulation time of hydrogel NPs (~200 nm) having Young's moduli of 10 kPa and 3000 kPa in mice was examined.³⁷ The soft NPs displayed a significantly higher persistence in the vasculature than their stiff counterparts especially in the first 2 h, and this difference became much less prominent at 4 h (**Figure 4a**). The different blood circulation performances of the soft and stiff NPs were attributable to their distinct phagocytosis profiles. The stiff NPs displayed a 3.5-fold higher uptake by J774 macrophages than the soft NPs over a 12 h period *in vitro*, and the higher macrophage capture of stiff NPs likely led to more rapid elimination.³⁷ Similar preferential macrophage uptake of stiffer NPs compared to the softer NPs was also reported in our recent study, wherein RAW264.7 murine macrophages exhibited significantly greater uptake of both unmodified and PEGylated stiff SNCs (9.7 GPa) than the soft ones (700 kPa).⁴⁷ Macrophage sequestration is a major reason accounting for the removal of NPs during circulation, and the lower macrophage uptake of soft NPs is likely due to their

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3 ability to deform upon the forces exerted by macrophage cells.³⁷ It has been reported
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5 that the extent of phagocytosis can potentially be decreased when the NP becomes
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7 elongated or stretched.⁵⁵
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11 The sieving effect of the biological filtration systems (e.g., lungs and spleen) represents
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13 another important mechanism responsible for NP removal. The blood circulation half-
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15 lives of hydrogel NPs (~120 nm) in mice decreased from 19.6 h to 9.1 h as their
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17 Young's moduli increased from 18 kPa to 1350 kPa (**Figure 4b**).³⁶ The longer
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19 circulation time of the softer NPs was attributed to its superior deformability, allowing
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21 them to pass through filters with a pore size smaller than its diameter without losing
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23 structural integrity (**Figure 4c**). In comparison, the stiffer NPs failed to pass through
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25 the filter. This sieving effect, as will be elaborated in next section, also applies to spleen
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27 which acts as a filter for purifying blood. Due to their compromised deformability,
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29 stiffer NPs normally exhibit more significant spleen accumulation than softer NPs,
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31 which in turn shortens their blood circulation time.
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38 Overall, soft NPs exhibit longer blood circulation than their stiff counterparts due to
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40 their lower macrophage capture and the ability to avoid the removal by biological
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42 filtration systems. This characteristic to some extent reflects the defending mechanisms
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44 against foreign substances and pathological tissues, considering that most endogenous
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46 substances in bloodstream (e.g., health red blood cells (RBCs)) possess very low
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48 stiffness, whereas pathological cells (e.g., diseased and aging RBCs) and foreign
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50 matters (e.g., viruses) are relatively stiffer.
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Figure 4. Effect of the mechanical property of nanoparticles on their blood circulation. (a) Circulation data of soft (10 kPa) and stiff (3000 kPa) hydrogel nanoparticles over 12 h. %ID represents the percentage of injected nanoparticle dose remain in blood circulation. Inset highlights the data from 0-2 h.³⁷ (b) Circulation profiles of poly(carboxybetaine) (pCB) hydrogel nanoparticles with varying mechanical properties over 72 h, with the pCB concentration from 2% to 15% corresponding to Young's moduli from 180 kPa to 1350 kPa.³⁶ (c) Stiff (1350 kPa) and soft (260 kPa) hydrogel nanoparticles with a mean hydrodynamic size of 250 nm to pass filters with a pore size of 220 nm. Schemes show that the stiff nanoparticles cannot pass the filter slits while the soft nanoparticles can deform and pass through the slits without losing their structural integrity.³⁶

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Biodistribution and tumor targeting

Biodistribution describes the spatiotemporal localization of NPs in the tissues and organs (e.g., lungs, kidney and spleen, etc.) of animals or human. Broadly speaking, the accumulation of NPs in solid tumors is within the scope of biodistribution. In fact, NPs'

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3 biodistribution, blood circulation, and tumor accumulation are three closely associated
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5 biological processes. Understanding the distribution profiles of NPs in different organs
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7 and tissues will shed light on the mechanisms involved in their clearance from the
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9 bloodstream by the mononuclear phagocyte system (MPS), which in turn determine
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11 their tumor targeting ability. As such, the effect of the physicochemical properties of
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13 NPs on their biodistribution has been extensively explored to promote the tumor-
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15 targeted delivery of NPs while reducing their off-target deposition, which are
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17 considered the grand challenges for NP-based drug delivery.^{1,56}
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23 As demonstrated by **Figure 4b** in the last section, the *in vivo* circulation time of
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25 hydrogel NPs reduced from 19.6 h to 9.1 h as their Young's moduli increased from 18
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27 kPa to 1350 kPa. To elucidate how these NPs were cleared from bloodstream, their
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29 biodistribution was examined (**Figure 5a**). At 48 h after intravenous injection, both the
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31 stiff and soft NPs were mainly found in livers and spleens, which are the major MPS
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33 organs responsible for the clearance of foreign substances.³⁶ Moreover, the
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35 accumulation of softer and stiffer NPs in liver did not show significant difference,
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37 whereas the splenic accumulation of stiffer NPs was significantly higher than that of
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39 the softer NPs, exhibiting a negative correlation with their blood circulation times
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41 (**Figure 5b**). This observation indicates the critical role of spleen in the filtration of NPs
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43 in the bloodstream, which can be better understood by taking a closer look into the
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45 microanatomy of the splenic venous sinuses (**Figure 5c**). Briefly, splenic venous
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47 sinuses are lined with a layer of discontinuous endothelial cells organized in parallel
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49 and connected by stress fibers, and this special arrangement delimits narrow slits
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51 serving as a filtration system.^{17,57} When blood from the splenic red-pulp cords collects
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53 in the sinuses by passing through the narrow slits (shown by arrows), healthy RBCs
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55 that are mechanically flexible are able to deform and squeeze through the slits to retain
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in the bloodstream, whereas aging or pathological RBCs are stiffened so that they are trapped in the macrophage-rich red-pulp and ultimately destroyed.¹⁷ This mechanism has been proven to also apply to the splenic removal of intravenously injected NPs.¹⁷ We recently, for the first time, demonstrated how decreasing NP elasticity can reduce their splenic clearance and thus enhancing their tumor targeting.⁴⁷ SNCs (~200 nm) having Young's moduli of 704 kPa and 9.7 GPa were modified with poly(ethylene glycol) (PEG) and folic acid (FA)-PEG, and were injected into mice bearing SKOV3 tumor xenograft that overexpress folate receptors (FRs). The ratios of SNC accumulation in tumor-liver (**Figure 5d**) and tumor-spleen (**Figure 5e**) was examined for 72 h. The tumor-liver ratios of the stiff and soft SNCs did not show evident difference, while the tumor-spleen ratios of the stiff SNCs were much lower than that of their soft counterparts, implying a higher splenic clearance of the stiff SNCs and a lower tumor targeting efficiency. It is worth noting that at 24 h post-injection, the tumor-liver and tumor-spleen ratios of FA-PEG-modified stiff SNC were significantly higher than its PEG-modified counterpart, indicating an enhancing effect of FA-functionalization on the tumor targeting ability of stiff SNCs, which was, however, not observed for soft SNCs. This is likely due to that FA-conjugation promotes the FR-mediated cellular uptake of the stiff SNCs to a higher extent than the soft ones. At all other time points, FA-PEG-modified SNCs did not show higher tumor accumulation than their PEG-modified counterparts, which reflects a key hindrance to the clinical success of active tumor targeting: ligands on the surface of NPs can only enhance cell–NP interactions at a very close proximity (<0.5 nm) but cannot substantially alter the overall *in vivo* NP biodistribution.³

The above studies demonstrate the crucial role of biological filtration systems (e.g., splenic sinusoid) in the clearance of intravenously injected NPs. Soft NPs are able to

1
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3 squeeze through these barriers and retain in the blood circulation, whereas stiff NPs are
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5 prone to be trapped due to their poor deformability. Normally, this higher accumulation
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7 in the MPS organs including spleen and liver leads to shortened blood circulation time
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9 and inferior tumor accumulation of stiff NPs. However, different results have also been
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11 observed. For instance, the accumulation of soft hydrogel NPs (~200 nm, 10 kPa) in
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13 the spleen and lung was significantly higher than that of the stiff NPs (3000 kPa)
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15 (**Figure 5f**), which was attributed to the longer blood circulation of the soft NPs.³⁷ The
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17 hydrogel NPs were functionalized with anti-intercellular adhesion molecule (ICAM) as
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19 ICAM protein is overexpressed in spleen and lung. In this case, spleen cannot be simply
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21 considered as a biological filter but a target organ. Given the complicated results
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23 discussed above, more explorations and better understanding on the effect of the
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25 mechanical property of NPs on their *in vivo* biodistribution and tumor targeting are
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27 needed.
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Figure 5. Effect of the mechanical property of NPs on their biodistribution and tumor targeting. (a) Biodistribution of pCB hydrogel nanoparticles with varying mechanical property, with the pCB concentration from 2% to 15% corresponding to Young's moduli from 180 kPa to 1350 kPa.³⁶ (b) Circulation half-life and splenic accumulation of the pCB hydrogel nanoparticles.³⁶ (c) Schema of a venous sinus located in the cords of the splenic red pulp.¹⁷ (d) Semi-quantitative analysis displaying the accumulation of stiff (9.7 GPa) and soft (704 kPa) silica nanocapsules between tumor and liver and (e) tumor and spleen. Values represent the ratios of integral fluorescence per unit mass (IF/g).⁴⁷ (f) Distribution of ICAM-conjugated stiff and soft hydrogel nanoparticles in various organs.³⁷

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Tumor penetration

After reaching the tumor tissue, most NPs accumulate at the periphery of the tumor mass. This inefficient tumor penetration of therapeutic NPs often leads to the difficulty in eradicating all tumor cells, thus suboptimal treatment outcomes and consequent cancer recurrence.^{58,59} Penetrating into the deep interior of a solid tumor is not trivial for NPs owing to a number of physiological barriers. Compared to normal tissues, tumor tissues are normally featured by a dense extracellular matrix due to their high content of collagen and lysyl oxidase.^{6,58} The interstitial fluid pressure inside tumors are also elevated because of the rapid cell proliferation and impaired lymphatic drainage.⁵⁸ These barriers prevent the diffusion of NPs into tumor interstitium. Researchers have attempted to improve NP penetration by optimizing their physiochemical properties such as size, shape and surface charge. Recently, the mechanical property of NPs has also been demonstrated to play a role in regulating their tumor penetration.

The tumor penetration of NPs is often investigated using tumor spheroids owing to their simple and easy preparation, their 3D architecture and spatial heterogeneity, and their similar chemistry and biology characteristics to tumor tissues.⁶⁰ By studying the interactions between FA-functionalized stiff/soft SNCs (~200 nm) and FR-positive SKOV3 tumor spheroids, we demonstrated the superior tumor penetrating ability of the soft SNCs (704 kPa), which exhibited a penetration depth of around 80 μm in the tumor

spheroids (**Figure 6a**).⁴⁷ In comparison, the stiff SNCs (9.7 GPa) were mainly located at the surface of the spheroids although they exhibited higher overall cellular uptake than their soft counterparts. The different performances of the soft and stiff SNCs were attributed to their distinct flexibility and deformability. The soft SNCs were able to squeeze through the intercellular spaces and transport deeper into the tumor spheroids, while the stiff SNCs were unable to pass through the narrow interstitial space, therefore trapped at the peripheral area of the spheroids (**Figure 6b**).⁴⁷ Additionally, the poorer tumor penetration of the stiff SNCs might also be a result of their stronger cell interaction, leading to their immobilization at the exterior of the tumor spheroids.

The penetration study of polymer micelles (~80 nm) with Young's moduli of 165 kPa and 260 kPa in BxPC3 pancreatic cell tumor spheroids revealed that the stiff micelles penetrated into all layers of the spheroid (the diameter of tumor spheroids was ~350 μm) including the centre site (**Figure 6c**), whereas not much softer micelles was found in the inner layer of the spheroid.⁴⁴ In another case, polymer-lipid NPs (~200 nm) with a moderate elasticity (50 MPa) exhibited a greater tumor permeability in BxPC3 and human pancreatic star cell (HPSC) multicellular spheroids when compared to their soft (5 MPa) and rigid (110 MPa) counterparts.³² The fluorescence signal of the semi-elastic NPs could be detected inside the tumor spheroids at a scanning depth of 70 μm , whereas the fluorescence signals of the soft and rigid NPs only occurred at the marginal area of the tumor spheroids at a scanning depth of 30 μm (**Figure 6d**). The stronger tumor penetration of the semi-elastic NPs was attributed to their enhanced diffusivity in biological hydrogels (e.g., mucus) both *in vitro* and *in vivo*. As elucidated by super-resolution microscopy and molecular dynamics (MD) simulation results, the semi-elastic NPs deformed into ellipsoids and performed rotational motion in biological hydrogels.³² This alteration in shape led to a higher aspect ratio, which boosted the

diffusivity of the NPs. The very soft and stiff NPs, however, either deformed excessively or retained their original shape in biological hydrogels, thus exhibiting weaker diffusivity.

The inconsistent results discussed above reflect the complex effect of NP mechanical property on tumor penetration, which might stem from the distinct experimental settings in current studies. For example, big gaps exist between the mechanical property of NPs fabricated in individual studies (**Figure 3**). By comparing the tumor penetration behavior of NPs having Young's moduli of 165 kPa and 260 kPa, one may develop a conclusion that a higher elasticity is favorable for the tumor penetration of NPs. However, 260 kPa is still far softer than real stiff materials. As the elasticity increases, NPs with a moderate elasticity may turn out to have better tumor penetrating ability than the softer and the stiffer ones. Another major limitation is that many key *in vivo* physiochemical settings are oversimplified in tumor spheroid models, such as the interstitial fluid flow and pressure, the stiffness of the tumor tissue, and the component of extracellular matrix, etc. The absence of precise control of these parameters can lead to poor representative of what really happens *in vivo*. Designing microfluidic devices that better mimic the *in vivo* conditions by integrating tumor spheroids with necessary tumor microenvironments (e.g., flow, pressure, tumor stiffness and permeability) may be a way to address this issue.^{61,62}

Figure 6. Effect of the mechanical property of nanoparticles on tumor penetration. (a) Fluorescence intensity distribution of stiff (9.7 GPa) and soft (704 kPa) silica nanocapsules across tumor spheroids at a scanning depth of 150 μm and (b) schematic illustration showing the penetration of the stiff and soft silica nanocapsules in the tumor spheroids.⁴⁷ (c) Distribution of soft and stiff hydrogel micelles in fluorescently-labelled tumor spheroids; blue: DAPI (nuclei), green: micelles, purple: DiD (cell membrane).⁴⁴ (d) Soft (5 MPa), semi-elastic (50 MPa) and hard (110 MPa) polymer–lipid nanoparticle penetration into tumor spheroids. Z-stack images were obtained starting from the top to the center of the spheroid at an interval of 20 μm . Scale bars = 50 μm .³²

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Interaction with cancer cells

The efficient internalization of drug-loaded NPs by tumor cells is a key prerequisite for successful drug delivery, hence great efforts have been dedicated to explore the design principles of NPs with enhanced cancer cell uptake. Despite that the majority of relevant studies report that stiff NPs display higher macrophage internalization than their soft counterparts, there remains a great discrepancy in the literature regarding the effect of NP elasticity on cancer cell uptake.

Many studies claim that NPs with higher elasticity are more favorable for internalization by cancer cells. By using PEGylated polymer–lipid NPs (~40 nm) with Young's moduli of 0.76 GPa and 1.2 GPa, the stiff NPs showed higher non-specific uptake by Hela cells compared to the soft NPs (**Figure 7a**).³³ As a result, doxorubicin and combretastatin A4 dual-drug loaded stiff NPs displayed greater cytotoxic effect than both free drug and drug loaded soft NPs. MD simulations reveal that the reduced cellular uptake of the soft NPs stemmed from their deformation during internalization, which raised the energy level required for their complete cell membrane wrapping (**Figure 7b**).³³ Similar MD simulation results have also been reported elsewhere.^{38,39} Interestingly, there are increasing evidences that the cellular uptake difference between the stiff and soft NPs can be magnified by modifying other characteristics such as surface chemistry. Hydrogel NPs (~200 nm) with Young's moduli of 10 kPa and 3000 kPa were studied for their uptake by 4T1 epithelial tumor cells.³⁷ When the surface of NPs was not modified, no statistical difference between the uptake of stiff and soft NPs was observed at short time points (≤ 4 h), and the stiff NPs were only bound to or internalized by 4T1 cells greater than their soft counterparts at 8 and 12 h (**Figure 7c**). Surface modification with anti-ICAM antibody enhanced the uptake of both stiff and

soft NPs by 4T1 cells that express ICAM receptors on cell surfaces. However, this enhanced uptake was more significant for the stiff NPs, with their uptake becoming statistically higher than that of the soft NPs at time points of 2 h, 4 h, 8 h and 12 h (**Figure 7d**).³⁷ These results indicate an interdependent effect between the mechanical property and surface property of NPs on determining their cancer cell uptake, which is in accordance with our recent studies. By studying the uptake of SNCs (~200 nm) having Young's moduli of 704 kPa and 9.7 GPa by FR-positive SKOV3 cancer cells, we found that the PEG-modified stiff and soft SNCs displayed similar non-specific cellular uptake, whereas the FA-PEG-modified stiff SNCs exhibited enhanced receptor mediated uptake by SKOV3 cells which overexpressed FA receptors on their surfaces, to a significantly higher extent than the soft ones (**Figure 7e**).⁴⁷ Mechanical property has also been demonstrated to work in tandem with the size of NPs to affect their cancer cell uptake. Stiff (260 kPa) and soft (165 kPa) PEG-PLA micelles having a diameter of ~17 nm showed no significant difference in the number of NPs adhered to A375 cancer cells (**Figure 7f**).⁴⁴ However, increasing the diameters of the micelles to ~40 nm and ~75 nm increased the cellular binding capacity of the stiff micelles much more significantly than that of the soft micelles, which consequently influenced their cellular internalization efficiency.

There are also studies wherein soft NPs display higher tumor cellular uptake than stiff NPs. For example, PEG-modified solid and hollow silica NPs (~300 nm) with Young's moduli of 233 MPa and 47 MPa, respectively, were studied for their uptake in MCF-7 breast cancer cells, and their morphological transition during cellular internalization was observed using TEM.⁴⁶ The soft SNCs could easily enter MCF-7 cells *via* a spherical-to-oval morphological change (**Figure 7g**), resulting in a 26-fold higher cellular uptake than their stiff counterparts which remained spherical during the

internalization. As a result, MCF-7 cells co-incubated with the DOX-loaded soft silica NPs showed higher cytotoxicity compared to the stiff NPs. In another case, the uptake of hydrogel NPs (~800 nm) by HepG2 cancer cells declined as their Young's moduli increased from 15 kPa to 156 kPa, which was attributed to the different uptake mechanisms involved in the endocytosis of stiff and soft NPs.³⁵ The softest NPs were mainly internalized *via* macropinocytosis, whereas the stiffest NPs largely adopted clathrin- and caveolae-mediated pathways, as well as macropinocytosis to enter the cells. The different endocytosis mechanisms involved in the cellular uptake of stiff and soft NPs have also been reported for liposome-based NPs. The uptake of soft liposomes (~160 nm, 45 kPa) by MDA-MB-231 and MCF-7 cancer cells was *via* both endocytosis and membrane fusion (**Figure 7h**), whereas the stiff hydrogel-lipid NPs (~160 nm, 19 MPa) could only enter the cells through endocytosis.⁴⁵ When membrane fusion, which requires less energy than endocytosis, was adopted by liposomes as a predominant way of entering the cells, softer liposomes displayed significantly higher cancer cellular uptake than their stiff counterparts.

Although it is clear that the cancer cell uptake of NPs can be modulated by modifying their mechanical property, it remains challenging to paint a complete picture about how the elasticity of NPs affects their interactions with cancer cells. A main reason for this is the heterogeneity in different cancer cells and the associated tumor microenvironments. The inconsistent experiment findings may also stem from the coupling effect of NP elasticity with other parameters such as size and surface chemistry. As discussed above, cellular internalization behaviors of stiff and soft NPs can be dramatically affected by altering their sizes or by functionalizing with targeting ligands. Another important reason is that the NPs used in most current studies possess a relatively narrow range of Young's modulus with considerable gaps between different

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3 systems (**Figure 3**), leading to inconsistent results. To address this issue, NP systems
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5 with a broad elasticity range from very soft (Pa) to very stiff (GPa) should be used to
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7 obtain a full spectrum result.
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11 Computational simulation is a powerful tool to explore the mechanisms behind the
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13 cellular adhesion and internalization of elastic NPs. For clathrin- and caveolin-
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15 independent endocytosis, the cellular uptake of NPs can be modelled by simple
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17 membrane wrapping based on the Helfrich–Canham membrane theory,⁶³ on which
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19 continuum scale theoretical analysis indicates that stiff NPs are more energetically
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21 favorable to full membrane wrapping than their soft counterparts, and this effect
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23 gradually becomes less significant as the NP stiffness increases.³⁹ Similarly, as
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25 previously discussed, MD simulation also suggests a faster full wrapping of stiff NPs
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27 than the soft ones by simple membrane (**Figure 7b**).³³ In real situations, however, the
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29 cellular uptake of NPs may not only involve the lipid membrane. As another membrane
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31 deformation process adopted normally by receptor-mediated cellular uptake, clathrin-
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33 mediated endocytosis not only requires the substantial membrane deformation as in the
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35 simple membrane wrapping, but also involves the adaptor protein-induced spontaneous
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37 curvature and actin-mediated force on budding. Recently, theoretical models at the
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39 continuum scale have been developed to describe clathrin-mediated endocytosis
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41 incorporating protein-induced heterogeneities in the deformed membrane.^{64,65} However,
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43 as the presence of NPs and the feature of NP-membrane interactions are not considered
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45 in these models, they are only valid for the clathrin-mediated endocytosis of NPs with
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47 radii smaller than that of the clathrin scaffold. For relatively larger NPs, depending on
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49 the stiffness ratio between the engulfed NPs and the self-assembled clathrin scaffold,
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51 the mechanically compressive interplay between the NPs and clathrin-coated
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53 membrane either squeezes the soft NPs to induce substantial NP deformation or
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3 expands the clathrin scaffold to accommodate the NPs, thus influencing the entire
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5 endocytic process. Other challenges remaining to be tackled include modelling the
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7 scission of clathrin-coated vesicles and endocytic kinetics such as the deterministic
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9 receptor diffusion and stochastic receptor–ligand binding and coat assembly. As the
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11 remodelling of the actin-based network and explicit consideration of its interaction with
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13 the lipid membrane are not fully established in MD simulations and is extremely
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15 computationally expensive and time-consuming, existing MD modellings in literature
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17 on the cellular uptake of elastic NPs are restricted to the cases of simple membrane
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19 wrapping.^{40,66,67} More sophisticated MD approaches integrating the lipid membrane and
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21 associated components remodelling are yet to be developed.
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Figure 7. Effect of the mechanical property of nanoparticles on their interactions with cancer cells. (a) Confocal fluorescent images showing the uptake of stiff (1.2 GPa) and soft (0.7 GPa) polymer–lipid nanoparticles by HeLa cells. co-localization of the PLGA core (red) and the lipid layer (green) shows a yellow collar, scale bar = 5 μm ; and (b) MD simulations showing the slower cell membrane wrapping of the soft nanoparticles

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3 compared to the stiff nanoparticles.³³ (c) Cellular uptake of bare and (d) ICAM-
4 conjugated soft (10 kPa) and stiff (3000 kPa) hydrogel nanoparticles by 4T1 cells at
5 various time points.³⁷ (e) The uptake of PEG- and FA-PEG-modified stiff (9.7 GPa)
6 and soft (704 kPa) silica nanocapsules by SKOV3 cells.⁴⁷ (f) Adhesion of soft (160
7 kPa) and stiff (260 kPa) hydrogel micelles with different sizes to A375 cells.⁴⁴ (g) TEM
8 images (upper 1-4) and schematic illustration (bottom 1-4) of the uptake process of soft
9 silica nanocapsules (47 MPa) by MCF-7 cells, with arrows indicating the cell
10 membrane. Scale bars = 100 nm.⁴⁶ (h) Schematic illustration showing that soft (45 kPa)
11 liposomes enter cells *via* membrane fusion (predominant) and endocytosis (inferior),
12 while hard polymer–lipid nanoparticles (19 MPa) enter cells *via* only clathrin-mediated
13 endocytosis.⁴⁵

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SUMMARY AND PERSPECTIVES

NP-based targeted cancer drug delivery depends upon a well-orchestrated series of biological events that requires synergistic effects of the physicochemical properties of NPs to overcome key biological barriers to drug delivery in cancer. This review highlights the impacts of the mechanical property of NPs on targeted cancer drug

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3 delivery through regulating their blood circulation, biodistribution, tumor penetration
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5 and tumor cell uptake. Compared to stiff NPs, soft NPs normally display less
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7 macrophage uptake and removal by biological filtration systems (e.g., splenic venous
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9 sinuses) owing to their superior deformability, leading to longer blood circulation times
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11 and higher tumor accumulation. This may to some extent explain why most currently
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13 commercialized anti-cancer drug delivery systems are based on soft materials (e.g.,
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15 liposome and albumin NP).⁶⁸ Despite these major findings, definitive conclusions on
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17 how NP elasticity affect their tumor penetration and interactions with cancer cells are
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19 still lacking, which is attributable to the overwhelming heterogeneity between different
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21 cancer cells and complex tumor microenvironments. The large gaps between the
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23 mechanical property of NPs in different studies, as well as the inconsistency in other
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25 physicochemical parameters (e.g., size, shape and surface modification) also contribute
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27 to the contradictory results of different studies. Therefore, the coupling effect between
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29 the mechanical property and other parameters of NPs on modulating nano-bio
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31 interactions needs to be more carefully elucidated in future studies. NP systems having
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33 a wide spectrum of mechanical properties should be used, and their mechanical property
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35 should be quantified using standardized methods to acquire comparable results. Up to
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37 now, studying cellular uptake pathways (using internalization inhibitors) and the energy
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39 costs involved in cellular internalization (using computational simulation) of stiff and
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41 soft NPs remain the main methods for exploring mechanisms governing mechanically
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43 regulated NP–cell interactions. Other important aspects such as the effects of NP
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45 mechanical property on the formation of protein corona, and on cell signalling pathways
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47 (using the emerging omics technique),⁶⁹ which affect NP cellular uptake, should also
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49 be systematically studied. This would require the efforts of multidisciplinary
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51 communities including nanotechnology, biophysics, cell biology and mechanical
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3 engineering. A more complete understanding of these topics would prompt the
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5 development of mechanically engineered NPs for superior targeted drug delivery. For
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7 example, NPs that are very soft during circulation for improving blood circulation time
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9 and consequently tumor accumulation, but then alter their elasticity by responding to
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11 tumor microenvironment or external stimulation for enhanced cancer cell
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13 internalization.
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30 **Author Contributions**

31
32 The manuscript was written through contributions of all authors. All authors have given
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34 approval to the final version of the manuscript.
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