Mechanical Immunoengineering of T cells for Therapeutic Applications

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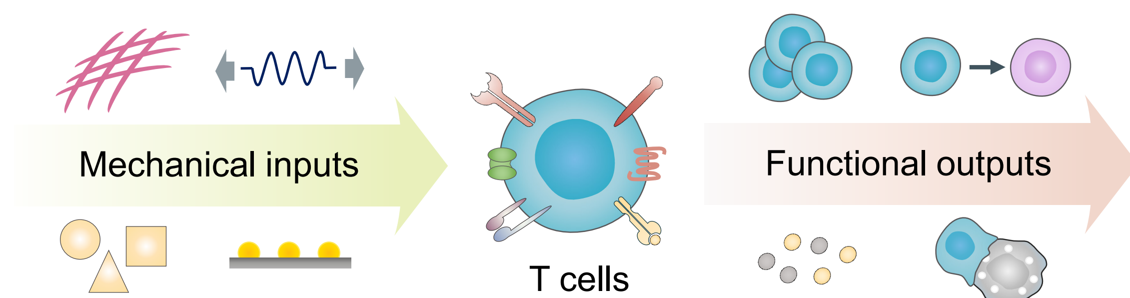
Conspectus

T cells, a key component in adaptive immunity, are central to many immunotherapeutic modalities aimed at treating various diseases including cancer, infectious diseases, and autoimmune disorders. The past decade has witnessed tremendous progress in immunotherapy, which aims at activating or suppressing the immune response for disease treatments. Most strikingly, cancer immunotherapy has led to curative responses in a fraction of patients with relapsed or refractory cancers. However, extending those clinical benefits to a majority of cancer patients remains challenging. In order to improve both efficacy and safety of T cell-based immunotherapies, significant efforts have been devoted to modulating biochemical signals to enhance T cell proliferation, effector functions, and longevity. Such strategies include discovery of new immune checkpoints, design of armored chimeric antigen receptor (CAR)-T cells, and targeted delivery of stimulatory cytokines.

Despite the intense global research efforts in developing novel cancer immunotherapies, a major dimension of the interactions between cancer and the immune system, its biomechanical aspect, has been largely underappreciated. Throughout their lifecycle, T cells constantly survey a multitude of organs and tissues and experience diverse biomechanical environments, such as shear force in the blood flow and a broad range of tissue stiffness. Furthermore, biomechanical properties of tissues or cells may be altered in disease and inflammation. Biomechanical cues,

1 including both passive mechanical cues and active mechanical forces, have been shown to
2 govern T cell development, activation, migration, differentiation, and effector functions. In
3 other words, T cells can sense, respond to, and adapt to both passive mechanical cues and active
4 mechanical forces.
5
6 Biomechanical cues have been intensively studied at a fundamental level but are yet to be
7 extensively incorporated in the design of immunotherapies. Nonetheless, the growing
8 knowledge of T cell mechanobiology has formed the basis for the development of novel
9 engineering strategies to mechanically modulate T cell immunity, a nascent field that we
10 termed "mechanical immunoengineering". Mechanical immunoengineering exploits
11 biomechanical cues (e.g., stiffness and external forces) to modulate T cell differentiation,
12 proliferation, effector functions, etc. for diagnostic or therapeutic applications. It provides an
13 additional dimension, complementary to traditional modulation of biochemical cues (e.g.,
14 antigen density and co-stimulatory signals), to tailor T cell immune responses and enhance
15 therapeutic outcomes. For example, stiff antigen-presenting matrices have been shown to
16 enhance T cell proliferation independently of the intensity of biochemical stimulatory signals.
17 Current strategies of mechanical immunoengineering of T cells can be categorized into two
18 major fields including passive mechanical cue-oriented and active force-oriented strategies. In
19 this Account, we first present a brief overview of T cell mechanobiology. Next, we summarize
20 recent advances in mechanical immunoengineering, discuss the roles of chemistry and material
21 science in the development of these engineering strategies, and highlight potential therapeutic
22 applications. Finally, we present our perspective on the future directions in mechanical
23 immunoengineering, and critical steps to translate mechanical immunoengineering strategies
24 into therapeutic applications in the clinic.
25

26 **Conspectus graphic**



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1 **Key references**

2 • Basu, R.; Whitlock, B. M.; Husson, J.; Le Floc'h, A.; Jin, W.; Oyler-Yaniv, A.; Dotiwala,
3 F.; Giannone, G.; Hivroz, C.; Biais, N.; Lieberman, J.; Kam, L. C.; Huse, M. Cytotoxic T
4 Cells Use Mechanical Force to Potentiate Target Cell Killing. *Cell* **2016**, *165*, 100–110.[1]
5 *This study demonstrated that enhancing T cell endogenous force potentiated T cell*
6 *cytotoxicity against tumor cells.*

7 • Lambert, L. H.; Goebrecht, G. K. E.; De Leo, S. E.; O'Connor, R. S.; Nunez-Cruz, S.; Li,
8 T. De; Yuan, J.; Milone, M. C.; Kam, L. C. Improving T Cell Expansion with a Soft Touch.
9 *Nano Lett.* **2017**, *17*, 821–826.[2] *This study illustrated that simply modulating particle*
10 *stiffness significantly improved T cell expansion as compared to a well-established T cell*
11 *stimulator (anti-CD3 and anti-CD28 antibody-coated Dynabead®).*

12 • Majedi, F. S.; Hasani-Sadrabadi, M. M.; Thauland, T. J.; Li, S.; Bouchard, L.-S.; Butte, M.
13 J. T-Cell Activation Is Modulated by the 3D Mechanical Microenvironment. *Biomaterials*
14 **2020**, *252*, 120058.[3] *This study discovered that T cells responded to 3D mechanical*
15 *environment by using an alginate-based 3D scaffold with consistent microporosity and*
16 *tunable stiffness.*

17 • Lei, K.; Li, T. T Cell Force-Responsive Delivery of Anticancer Drugs Using Mesoporous
18 Silica Microparticles. *Mater. Horizons* **2020**, doi:10.1039/D0MH01285H.[4] *This study*
19 *demonstrated that T cell force can be utilized to trigger specific release of anticancer drugs*
20 *using a DNA-capped mesoporous silica microparticle system.*

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23 **1. Introduction**

24 T cells, an important factor of adaptive immunity, are responsible for cellular responses against
25 pathogens and malignant cells. T cells are identified by T cell receptor (TCR)/cluster of
26 differentiation 3 (CD3) complexes located on the cell surface. TCR triggering by antigen
27 peptide-bound major histocompatibility complex (pMHC) results in intracellular signaling
28 mediated by cytoplasmic portions of CD3 for T cell activation. Given their core role in
29 immunity, T cells lie at the heart of various immunotherapeutic modalities against cancer,[5]
30 infectious diseases,[6] and autoimmune disorders.[7] For instance, checkpoint blockades that
31 reactivate exhausted or dysfunctional T cells[8,9] and adoptive cell therapies (ACT), in particular

1 chimeric antigen receptor (CAR)-T cells,[5,10] have been approved recently by the Food and Drug
2 Administration (FDA) for the treatment of a variety of cancers that are non-responsive to
3 traditional forms of cancer therapy (e.g., chemotherapy and radiation therapy). However, only
4 a small fraction of cancer patients respond to current immunotherapies.

5 Immunoengineering of T cells could improve the potency and/or safety of immunotherapies,
6 potentially benefiting more patients. One such example is ACT adjuvanted by supporting
7 cytokines, which are secreted by genetically-engineered T cells or released from stimuli-
8 responsive biomaterials specifically in the tumor environment.[11–13] To date, attempts to
9 improve immunotherapy have focused predominantly on regulating biochemical traits of T
10 cells or their surrounding biochemical microenvironment (we term those strategies biochemical
11 immunoengineering).

12 On the other hand, biomechanical cues, which represent another major dimension of the
13 relationship between T cells and their environment, have been largely underappreciated. Indeed,
14 across the multiple stages of T cell immunity, T cells experience myriad forces and encounter
15 environments with diverse mechanical properties. T cells patrol the body through the
16 circulatory system, where they are exposed to shear stress induced by blood flow. The resulting
17 shear forces acting on T cells have been shown to promote transmigration through endothelial
18 layer via force-induced affinity maturation of integrins on T cells.[14] Following extravasation,
19 T cells encounter tissues and organs with a wide range of mechanical properties. Moreover,
20 tumor and fibrotic tissues typically exhibited higher stiffness compared to healthy tissues.[15]

21 Accumulating evidence shows that biomechanical cues are essential for T cell functions and
22 enhance T cell sensitivity to biochemical signals.[1,16,17] In this regard, integrating biomechanical
23 principles into the design of the next generation of T cell-based immunotherapies may enhance
24 efficacy and lower toxicity by improving specificity. The growing understanding of T cell
25 mechanobiology provides a solid basis to exploit mechanical cues to modulate T cell immunity
26 for therapeutic applications, a new field we term "mechanical immunoengineering" (Figure 1).
27 In this account, we review recent progress in T cell mechanobiology and strategies of
28 mechanical immunoengineering of T cells and present them in the context of therapeutic
29 applications. We envision that mechanical immunoengineering will develop into an important
30 methodology that is complementary to biochemical immunoengineering, and ultimately
31 improve the response rate of immunotherapies.

32

2. Mechanobiology of T cells

T cell mechanobiology has been thoroughly reviewed in several excellent articles.[18–22] Instead of giving a comprehensive review of the field, here we present a brief summary of T cell mechanosensing and its underlying mechanobiology.

T cell activation requires three elements: 1) antigen-specific stimulation signal through the TCR/CD3 complex; 2) costimulatory signal through CD28; and 3) survival or differentiation signal from autocrine or paracrine cytokines. TCR signaling has been classically viewed as a purely biochemical process initiated upon recognition of pMHC. In recent studies, the TCR, upon binding to agonist pMHC, has been shown to exhibit a "catch bond" behavior under mechanical tension. Catch bond is a specific type of non-covalent receptor-ligand interaction displaying prolonged bond lifetime upon tensile force applied to the receptor-ligand axis.[23–25] Catch bond formation between TCR and pMHC enables T cells to discriminate between agonist and antagonist pMHCs.[26] A recent study further showed that catch bond formation is important for negative selection of T cells in the thymus.[27] Moreover, mechanical force directly acting on the TCR or CD3 can trigger TCR signaling likely due to force-induced allosteric changes in the TCR/CD3 complex,[28,29] showing evidence that the TCR/CD3 complex itself is a mechano-sensor.

TCR binding to pMHC upon contact with a cognate antigen-presenting cell (APC) induces a large-scale spatial reorganization of receptor-ligand complexes into a specialized cell-cell junction called immunological synapse (IS), which orchestrates sustained TCR engagement and subsequent T cell functions such as cytokine production and clonal expansion.[30,31] A canonical IS features a "bull's eye" pattern with central supramolecular activation cluster (cSMAC) mainly containing TCR/pMHC clusters surrounded by a ring of lymphocyte function-associated antigen 1 (LFA-1)/intercellular cell adhesion molecule-1 (ICAM-1) complexes termed peripheral supramolecular activation cluster (pSMAC).[22] LFA-1, a member of the integrin family exclusively expressed on leukocytes, is of particular interest to T cell mechanobiology as recent evidence shows that LFA-1 is a mechano-sensor and a key regulator of IS formation.[32] During T cell activation, LFA-1 matures from an inactive, bent conformation with very low affinity to its cognate ligand ICAM-1 on APCs to an intermediate state with higher affinity. Mechanical tension on the LFA-1/ICAM-1 pair arising from F-actin centripetal flow at the IS further transforms LFA-1 into an active, extended conformation with high ligand affinity promoting T cell adhesion and priming.[32]

T cells also actively exert mechanical forces.[16,33–36] Both TCR and LFA-1 are coupled to the T cell cytoskeleton, effectively connecting the cell intracellular domain to external surfaces and relaying cytoskeletal forces to the surface of the APC or target cell. Lowering these cytoskeletal forces via cytoskeleton inhibition is associated with significant reduction in T cell activation events such as calcium influx and interleukin-2 (IL-2) production.[16,37] Calcium influx can be partly rescued by external cyclical forces applied to the TCR.[16] These observations suggest that T cells are a major contributor to the mechanical forces required for TCR triggering. Mechanical forces exerted by cytotoxic T lymphocytes (CTLs) at the IS interface with target cells, such as cancer cells, enhance perforin-based pore formation on the membrane of target cells, and potentiate killing of target cells.[1] A recent study further demonstrated that F-actin-rich protrusions of CTLs were required for this synaptic force exertion and cytotoxicity against target cells.[38]

The fundamental studies discussed above provide a brief insight into the mechanical life of T cells. Bioengineering strategies derived from these findings and aimed at modulating T cell immunity can be broadly classified according to their physical nature into two fields: passive mechanical cue-oriented and active force-oriented mechanical immunoengineering of T cells (Figure 1). In general, the same mechanosensitive receptors (e.g., TCR and LFA-1) are involved in both passive and active mechanical stimulations of T cells. In the following sections, recent advances in both fields will be discussed in detail with focus on therapeutic applications.

3. Passive mechanical cues for mechanical immunoengineering of T cells

Passive mechanical cues refer to mechanical properties of the microenvironment encountered by cells, such as extracellular matrix (ECM) stiffness and spatial organization of its networks including fibronectin and collagen fibers. These cues exist throughout the body and are indispensable for guiding the cellular responses (e.g. migration and differentiation) of various cell types, such as fibroblasts and stem cells.[39,40] However, less is known about their effects on T cells.

For example, inflamed and tumor tissues display distinct ECM mechanical properties compared to healthy tissues. Over the course of inflammation, sentinel lymph nodes become up to 10 times stiffer than normal lymph nodes (~4 kPa).[41] In tumor tissues, collagen crosslinking contributes to ECM stiffening and promotes tumor progression.[42] At the cellular

level, maturation of dendritic cells (DCs), the most potent professional APC, results in a rise of their cell stiffness through cytoskeleton remodeling.[17] At the same time, they undergo drastic morphological changes and exhibit a stellate shape optimal for T cell stimulation.[43] In this section, we discuss the role of the substrate and cell stiffness as well as topography and ligand patterning, two intensively-studied categories of passive mechanical cues, in regulating T cell responses and their potential use in mechanical immunoengineering of T cells.

3.1. Substrate/cell stiffness

Stiffness is an inherent mechanical property of materials representing their resistance to deflection or deformation. Substrate stiffness is defined by its Young's modulus or elastic modulus (E). Polydimethylsiloxane (PDMS) elastomer and polyacrylamide (PA) hydrogel are two widely-used substrates to control stiffness experienced by cells in vitro, predominantly due to their non-degradable and bio-inert features that enable presentation of specific ligands with minimal non-specific biochemical interactions.[40]

Early studies identified that PDMS substrates with stiffness values of approximately E ~100 kPa induced the strongest activation of human or murine naïve T cells.[37,44] This stiffness-dependent activation was partly attributed to the myosin-mediated cell contractility. Interestingly, a recent study demonstrated that spreading area of T cells exhibit maximal spreading on PDMS substrates with a stiffness of ~5 kPa.[45] These results suggest that, within the range of 0-5 kPa, the enhanced T cell activation observed on stiffer PDMS substrates may be partially attributed to the increased amount of stimulatory ligands accessed by the cell due to its larger spreading area. However, why T cells prefer certain stiffness of PDMS substrate (~100 kPa) for optimal activation still requires further investigation.

In light of the stiffness-dependent activation of T cells, this mechanical parameter may be incorporated in the design of engineering strategies to improve T cell manufacturing for cell therapies. Current ex vivo expansion of T cells for clinical applications mainly relies on a synthetic microbead system (Dynabead®) modified with anti-CD3 and anti-CD28 antibodies on its surface. This system is, however, made of polystyrene (PS), an extremely stiff material with E in the GPa range, several orders of magnitude higher than physiological stiffness. Using PDMS microbeads (E~8 MPa) to present stimulatory signals to human primary T cells resulted in enhanced expansion of functional T cells compared to Dynabeads®.[2] It is worth noting that

this PDMS microbead system may be further improved by using lower stiffness values as optimal T cell activation was achieved on a PDMS substrate with stiffness of 100 kPa.

Despite these promising results, the stiffness of PDMS typically ranges from 100 kPa to 10 MPa, which is substantially higher than physiological stiffness that T cells encounter in vivo, typically within 100 Pa to 100 kPa (except bone). Over the past years, in-depth studies of T cell responses to stiffness in a physiologically-relevant range (0.1-100 kPa) have been performed, generally using hydrogel substrates. In an elegant study, Hivroz's group designed an artificial antigen-presenting surface using PA hydrogels with stiffness values ranging from 0.5 to 100 kPa.[46] Within this range of stiffness, re-stimulated human $CD4^+$ T cells displayed increasing cell proliferation and inflammatory cytokine production with increasing stiffness. In another study, an alginate hydrogel-based system mimicking the stiffness changes of lymph nodes during inflammation (from 4 to 40 kPa) was developed.[3] This study demonstrated that priming of naïve murine $CD4^+$ T cells depends on the mechanical microenvironment in both 2D and 3D culture systems.

The intracellular mechanisms underlying the stiffness dependence of T cell activation is an active field of research. For example, gene expression profiling revealed an upregulation of hypoxia-inducible factor-1α (HIF1A), a transcriptional factor stimulating glycolytic metabolism, in T cells simulated on a substrate of 100 kPa.[46] Furthermore, the respiratory electron transport pathway involved in adenosine triphosphate (ATP) production was highly induced, suggesting a potential role of cellular metabolism in regulating T cell responses to stiffness.

Mechanosensing in T cells requires Yes-associated protein (YAP), a well-known transcriptional regulator in mechanotransduction.[41] In general, cells recognize increased stiffness via enhanced translocation of YAP from the cytosol into the nucleus. YAP knock-out T cells failed to recognize stiffness changes and displayed enhanced activation on a soft substrate (E ~4 kPa) compared to wild type T cells. [41] Interestingly, YAP was upregulated upon activation, but acted as a negative regulator of T cell activation. This finding is contrary to the usual role of YAP in adherent cells, where YAP promotes proliferation. YAP dampens T cell proliferation by restricting trafficking of Nuclear Factor of Activated T cells (NFAT), a known promoter of IL-2 expression, into the nucleus.[41] However, whether YAP itself contributes to T cell proliferation requires further investigation. This finding may provide a novel target to engineer optimal T cell responses by fine-tuning the mechanosensing properties of T cells.

While artificial antigen-presenting surfaces provide convenient tools to study T cell activation, they hardly recapitulate all aspects of a natural APC. To date, there has been a limited volume of research on how the stiffness of APCs or target cells shapes T cell responses. Cancer cells cultured on stiff surfaces adapt to their environment and show increased cellular stiffness as a result.[47] Recently, cancer cells on stiff substrates were shown to be more vulnerable to CTL-mediated cytotoxicity, suggesting that stiffness of target cells may play a role in the T cell responses.[1] Similarly, effector $CD4^+$ T cells produced more cytokines when stimulated by target cells cultured on stiffer substrate (Figure 2).[46] However, mechanistic studies are required to determine the extent to which stiffness of target cells influences T cell effector functions. More recently, Tello-Lafoz et al. discovered that increased cellular stiffness resulting from enhanced expression of myocardin-related transcription factor (MRTF) in metastatic cancer cells enhanced their susceptibility to CTL-mediated killing.[48] Sensing of this biomechanical vulnerability by T cells, a process termed “mechanical immunosurveillance”, offers a novel perspective on the biomechanical nature of T cell immunity, as well as a possible target for mechanical immunengineering of T cells.

Besides activation of effector T cells, there is scarce information about the role of stiffness in the induction of regulatory T cells (Tregs). Induction of murine Tregs was enhanced on a PDMS substrate with stiffness of ~100 kPa as compared to a non-physiological stiffness (~3 MPa). In contrast to effector T cell activation, interruption of myosin-mediated contractility using a Rho-associated kinase (ROCK) inhibitor increased Treg induction.[49] The mechanism underlying this surprising observation requires further in-depth investigation. The results suggest that stiffness may be an essential parameter to optimize ex vivo induction of Tregs for ACT against autoimmune diseases.[50]

Recently, attempts have been made to use the stiffness dependence of T cells to enhance cancer immunotherapy. Hickey et al. improved ex vivo expansion of antigen-specific murine $CD8^+$ T cells by leveraging a soft hyaluronic acid (HA) hydrogel (~0.5 kPa) presenting stimulatory signals. T cells expanded on the HA hydrogel showed improved tumor control and prolonged survival rate in mice compared to T cells expanded on a conventional tissue culture plastic (TCP). This effect is likely due to the enhanced proliferation of antigen-specific T cells (Figure 3).[51] Of note, T cell activation was reduced on HA hydrogels of higher stiffness, ~3 kPa (Figure 3B), which stands in opposition to previous studies. Although the authors did not give an explanation, this singular result may be caused by specific interactions between CD44 of T

1 cells and HA as CD44-mediated signaling is mechanosensitive.[52]

2 Despite considerable progress on understanding how stiffness affects T cell responses, it is still
3 challenging to study this effect in vivo, especially at the cellular level. Lysyl oxidase (LOX)
4 may be an interesting tool to modify tissue stiffness in vivo.[53] However, to the best of our
5 knowledge, no approach is available to control cell stiffness in vivo. In the future, efforts should
6 be dedicated to in vivo modulation of cell stiffness, which is critical to study mechanical
7 regulation of T cell responses in vivo.

8

9 **3.2. Topography and ligand patterning**

10 Upon maturation, DCs exhibit morphological changes characterized by dendrite structures,
11 which are important for migration to lymph nodes and the subsequent priming of T cells.[54,55]
12 This realization, along with improved understanding of the biological effects of topography,[39]
13 has stimulated research on topography as a novel parameter to design artificial APCs. For
14 example, Sunshine et al. evaluated T cell activation by pMHC/anti-CD28-conjugated
15 ellipsoidal microparticles compared to spherical microparticles.[56] The topography of
16 microparticles was controlled using a film-stretching method that enables constant particle
17 volume across various topographies. At similar antigen density, the ellipsoidal artificial APC
18 generated higher expansion of antigen-specific murine $CD8^+$ T cells in vitro and in vivo
19 compared to its spherical counterpart and resulted in enhanced therapeutic efficacy (Figure 4).
20 This result is largely attributed to increased contact frequency and area between the T cell and
21 ellipsoidal artificial APC. In a follow-up study, a similar trend was observed using nano-sized
22 ellipsoidal particles, which may have additional advantages, such as improved
23 pharmacokinetics upon intravenous injection and easier access to draining lymph nodes,
24 compared to ellipsoidal microparticles.[57]

25 Spatial proximity of TCR complexes is essential to TCR triggering.[58] Ligand nanopatterning
26 techniques have been widely used in the past decade to study the role of lateral ligand spacing
27 on T cell activation.[59,60] Multiple studies from Spatz's group showed that anti-CD3 antibody
28 lateral spacing should be below 60 nm for optimal activation. Above this threshold, TCR
29 signaling was abrogated, even if sufficient anti-CD3 molecules for T cell activation were
30 present in the contact area.[59,61,62] It is worth noting that these studies did not decouple the effect
31 of ligand spacing from ligand density. Intriguingly, the threshold for pMHC lateral spacing was

as large as ~150 nm,[63] suggesting other spatial requirements during TCR triggering in addition to CD3/ligand interactions.

In an elegant study reported by Cai et al. recently, ligand lateral spacing was set independently from the number of ligands accessible per cell.[60] A threshold of ~50 nm in lateral spacing of anti-CD3 was revealed in TCR triggering. This finding is consistent with the distance required for auto-phosphorylation of zeta-chain-associated protein kinase 70 (ZAP70) that is recruited to the TCR intracellular domain, a key step for TCR downstream signaling.[60] Knowledge gained in fundamental research can be leveraged to improve the expansion of pre-stimulated human $CD4^+$ T cells. Nanopatterned anti-CD3 antibody-coated surfaces with optimized lateral spacing outperformed conventional Dynabeads®,[62] suggesting that ligand nanopatterning may aid in designing more potent artificial APCs for ex vivo T cell expansion.

The spatial organization of the IS varies dramatically between different subtypes of T cells.[64] Motivated by these variations, efforts have been made to fabricate artificial ligand micropatterns and study their effects on T cell responses. Using microarrays, Dustin's and Groves' groups showed that TCR clusters mechanically confined in the IS periphery showed longer lasting TCR signaling compared to those in the center of an IS with a typical "bull's eye" structure.[65] As cSMAC has been shown to facilitate TCR degradation via endocytosis,[66] the peripheral confinement of TCR may augment TCR signaling by preventing TCR degradation. Irvine's group employed polymer-assisted photo-lithography to construct different anti-CD3-immobilized micropatterns which direct the spatial distribution of TCR signaling proteins at the IS.[67] Production of interferon-γ (IFN-γ), a pro-inflammatory cytokine, by murine $CD4^+$ T cells was specifically attenuated on annular micropatterns, while IL-2 secretion was at similar levels on both focal and annular micropatterns.[67] These observations suggest that TCR micropatterning plays a decisive role in guiding T cell functions, providing a possible mechanical cue to direct T cell responses.

The spatial organization of costimulatory ligand CD28 is another feature of IS formation, which is important in regulating T cell activation.[68] Typically, CD28 ligands form a ring-like structure surrounding the cSMAC region containing TCR/CD3 complexes. The CD28 pattern is important to retain the spatial proximity between TCR/CD3 and protein kinase C θ (PKCθ), a TCR downstream signaling molecule recruited by CD28.[68] To study the effects of CD28 patterning, Kam's group utilized microcontact printing to fabricate an anti-CD3/anti-CD28-colocalized central focal (COL) micropattern, as well as a segregation (SEG) layout with a

central focal anti-CD3 domain surrounded by four anti-CD28 dots that mimics the native pattern of an IS.[69,70] While murine CD4$^+$ T cells produced more IL-2 on SEG pattern than on COL pattern,[69] human CD4$^+$ T cells showed a reversed trend.[70] These findings not only provide insight into the design principle of ligand spatial organization for engineering T cell responses, but also emphasize the potential dissimilarity in mechanosensing between human and murine T cells. Interestingly, dissimilarities between murine Tregs and effector CD4$^+$ T cells in adhesion to micropatterns were also noticed.[71] This preference for distinct spatial ligand organization holds promise for selective activation of Tregs.

To date, investigations of the role of ligand patterning in regulation of T cell responses are still limited to in vitro studies. As a next step, it is crucial to validate these in vitro findings in animals. Tools for introducing ligand patterning in animals have been reported in investigations of stem cell adhesion[72] and could potentially be applied to study mechanical modulation of T cells.

4. Active forces for mechanical immunoengineering of T cells

Immunoreceptors, which connect the intracellular domain to the extracellular environment, experience a variety of mechanical forces as T cells patrol the body and affect their functions. The resulting tension on these receptors (e.g., TCR and LFA-1) is essential for effective outside-in signaling, which notably promotes T cell effector functions and inside-out signaling.[20] This tension originates, among others, from T cell motion, membrane undulations, and active cytoskeletal rearrangements. T cells continuously remodel their cytoskeleton and actively probe their environment using actin-rich protrusions which deform target cells and substrates.[38,73] They exert cytoskeletal forces through on their environment when they sense matrix stiffness[74] and during migration,[75] activation,[33,34] and killing.[1] Forces acting on immunoreceptors can also originate from external sources such as hydrodynamic forces and target cell cytoskeleton remodeling. These examples and those discussed earlier in this Account highlight the key role of active mechanical forces at every step of the T cell response. It is also apparent that strategies aiming at leveraging or modulating T cell endogenous forces or applying external force on immunoreceptors may be used to optimize T cell-based therapies.

4.1. Forces exerted by T cells

1 T cell mechanical force and its underlying actin remodeling during motility, activation,[16] and
2 effector functions[1] have been documented. In particular, mechanical forces at the interface
3 between T cells and stimulatory surfaces have been extensively characterized.[16,33,34]
4 Abrogation of these forces using cytoskeleton inhibitors such as Latrunculin A resulted in loss
5 of activation or effector functions.[1,16] Moreover, increased force exertion of phosphatase and
6 tensin homolog (PTEN)-deficient CTLs was shown to significantly enhance tumor cell killing
7 in vitro.[1] Huse's group demonstrated, through a series of in vitro studies, that while T cell force
8 was enhanced in PTEN-deficient T cells, other key biochemical aspects of their cytotoxicity,
9 such as granule polarization and release, were unaffected by PTEN suppression. A direct link
10 between increased force exertion and enhanced killing is still lacking due to technical
11 difficulties, but these results suggest that engineering T cells to exert higher forces may be a
12 viable strategy to enhance killing. One main limitation of this strategy lies in its target, PTEN,
13 which is involved in a variety of cellular processes, including cellular proliferation, survival,
14 growth, and motility.[76] Data from the Huse's group showed that PTEN-deficient CTLs have
15 reduced ability to control tumor size in vivo compared to wild type CTLs, owing to reduced
16 migration and homeostatic proliferation.[77]

17 Another force-enhancement strategy may be targeting cytoskeletal proteins. For example,
18 microtubules (MT) destabilization using nocodazole was shown to increase traction stresses of
19 Jurkat T cells.[78] However, the wide-ranging role of MT in T cells means that the disruption of
20 MT is likely to affect T cell functions. Indeed, nocodazole-treated human $CD4^+$ T cells
21 exhibited a shift towards an amoeboid phenotype and enhanced migration through complex 3D
22 environments in vitro, which may be beneficial to T cell infiltration in dense tumor tissues.[79]
23 On the other hand, nocodazole-treated T cells exhibited strongly reduced killing of target
24 cells.[80] Each specific cytoskeletal target should be thoroughly analyzed and its potential
25 evaluated according to the desired outcome, e.g. enhanced therapeutic efficacy.

26 T cell forces are triggered upon engagement of the TCR/CD3 complexes by pMHCs or anti-
27 CD3 antibodies. Our group recently introduced a novel drug delivery system where drug
28 release is induced by T cell force exertion upon TCR triggering.[4] This system is composed of
29 mesoporous silica microparticles loading with gemcitabine, an anticancer drug, whose release
30 is blocked by anti-CD3 antibody-conjugated DNA mechano-probes as gatekeepers on the
31 mesopores (Figure 5A). Upon TCR engagement, T cell forces unzip the DNA gatekeepers and
32 thus uncap the mesopores leading to release of encapsulated anticancer drugs (Figure 5A). The

T cell force-responsive delivery of anticancer drugs significantly enhanced killing of cancer cells both in vitro and in vivo (Figure 5B-D). This approach showcases how T cell force can be used to precisely control the release of a molecule of interest for enhanced therapeutic outcomes.

4.2. External forces applied on T cells

It is well established that the TCR is mechanosensitive.[28,29] Tension on the TCR-pMHC ligand pairs induces catch bonds, which exhibit maximal bond lifetime under forces of approximately 10 piconewton (pN).[26] This long-lived interaction is essential to trigger strong T cell activation. Using DNA linkers with defined breakage forces, Salaita's group showed that T cells transmit forces of 12-19 pN per TCR to initiate activation.[36] However, there is no consensus yet on the nature (constant vs. cyclic; normal vs. shear) and magnitude of the force required for optimal T cell activation. Interestingly, several studies showed that external forces on TCR can induce calcium influx in T cells.[16,26,28,29] Moreover, force applied on the TCR of LatA-treated cells could rescue calcium flux, suggesting that the cytoskeleton could be bypassed.[16]

The evidence presented here suggests that active mechanical forces may be used to recapitulate the mechanical conditions at the immune synapse during activation and engineer T cell responses. Therapeutic applications require a large number of cells which can be analyzed in vitro and subsequently tested in disease models. The majority of the elegant tools used in mechanobiology, such as magnetic tweezers, atomic force microscopy, and biomembrane force probe are intrinsically limited to single-cell studies and not suitable for large-scale experiments. We refer readers to several excellent articles on this topic.[20,81,82] Hence, in this section, we highlight key studies using external mechanical forces to engineer T cells for therapeutic purposes.

Formation of the IS following TCR ligation results in intense ligand reorganization, including TCR clustering.[22] This aspect is often overlooked in many artificial T cell activation systems, where anti-CD3 antibodies or pMHCs are immobilized and cannot be reorganized by T cells. Magnetic forces acting on superparamagnetic nanoparticles bound to TCRs were used to drive the receptors laterally towards one another, thus resulting in TCR clustering (Figure 6A).[83] TCR clusters in the presence of magnetic field were significantly larger than those in the absence of magnetic field, resulting in greater ex vivo T cell expansion and better tumor control

1 and mouse survival in vivo following adoptive transfer of expanded T cells (Figure 6B-D).
2 Recently, magnetic forces were also used to gently stir (250 rpm) T cells attached to magnetic
3 microparticles displaying anti-CD3 and anti-CD28 antibodies on their surface.[84] Impressively,
4 T cells expanded up to 12 times more under dynamic conditions compared to static conditions.
5 However, it is unclear whether this enhanced proliferation is the result of forces acting on TCR
6 and CD28 receptors or other factors, such as increased contact frequency between T cells and
7 beads. In vivo, T cell activation takes place in secondary lymphoid organs (SLO) where fluid
8 flow and changing tissue mechanics create a dynamic mechanical environment. The fluid
9 movement generated during stirring may mimic this aspect of SLO and enhance T cell
10 activation and proliferation. Interestingly, a commercially-available platform for
11 manufacturing of clinical-grade CAR-T cells (WAVE Bioreactor©) is performed on rocking
12 bioreactors to induced optimal mixing and gas transfer.[10]

13 Overall, these studies demonstrate that systems applying forces onto T cell populations globally
14 can be leveraged to optimize T cell activation and expansion, a key step in the manufacturing
15 of cell-based therapies.

16 An alternative strategy for stimulation of T cells using mechanical force relies on
17 optomechanical actuators,[85] which comprise nanoparticles that can absorb infrared light and
18 shrink upon illumination. The swift particle contraction can generate pN-range forces
19 transmitted through pMHC molecules to activate T cells. While the cell throughput is relatively
20 low, mainly due to the limited illumination area (micrometer range), this technique provides a
21 broad actuation frequency range along with high spatial resolution in a contactless manner.

22 Despite their promise for precise control of mechanical stimulation of T cells, optical systems
23 are limited in vivo due to the limited penetration of light through tissues (micrometers to
24 millimeters).[86] Ultrasound offers an interesting alternative as it can safely reach tissues at depth
25 up to several centimeters and ultrasound-based systems have been widely used in the clinic.[87]
26 In addition, microbubbles attached to the cell surface via receptors can be stimulated with low
27 frequency ultrasounds of 1-2 MHz at depths up to 5 centimeters.[88] Acoustic stimulation of
28 integrin-bound microbubbles on T cells successfully induced calcium influx through Piezo1
29 channels in T cells in vitro followed by translocation of NFAT transcription factor to the
30 nucleus (Figure 7A, B). T cells were engineered to express CD19-targeted CARs regulated by
31 NFAT, thus creating T cells genetically engineered to be specifically activated upon ultrasound
32 stimulation, i.e. a mechanogenetic system (Figure 7C, D). Therefore, spatiotemporal control of

CAR-T activation could be potentially achieved using external mechanical signals. Despite the novelty of this approach, the use of microbubbles coupled to T cells limits its application in vivo, as shear forces are likely to damage or burst the bubbles during T cell migration and extravasation.

5. Outlook

In the past decade, manipulation of T cell responses using biomechanical cues has emerged as a novel direction for immunotherapy. However, mechanical immunoengineering is still a nascent field with only a handful of in vivo studies in preclinical models. While these proof of concept studies have shown great promise, the future development and translation of mechanical immunoengineering approaches face challenges.

Our understanding of T cell responses to mechanical stimuli, the basis for developing novel mechanical immunoengineering strategies, is still in its infancy. For instance, pMHC, a natural TCR ligand, generates a more robust stiffness-dependent T cell response compared to anti-CD3 antibody.[17] However, most stiffness-dependent T cell behaviors were observed on anti-CD3-coated artificial surfaces. Understanding the key differences between pMHC and anti-CD3 may help engineer an APC with well-controlled signal strength. Critical and systematic comparison of the biomechanical behaviors of $CD4^+$ and $CD8^+$ T cells is still lacking. For example, naïve $CD4^+$ and $CD8^+$ T cells showed striking differences in mechano-sensitivity when activated on substrates with varying stiffness,[17] but the underlying mechanism remains elusive. In addition, T cell activation is more sensitive to alteration of the stiffness of target cells compared to artificial antigen-presenting substrates. The reason for this distinct difference in sensitivity remains unknown. Besides activation, T cell differentiation is key to initiating and maintaining a desirable T cell response for disease treatments.[89] Several recent studies showed that biomechanical cues can regulate T cell differentiation independently of the intensity of biochemical signals.[49,51] For example, lowering stiffness of HA hydrogel matrix enhanced induction of central memory-like human $CD8^+$ T cells under constant stimulatory ligand density.[51] However, the pathways or transcription factors involved in mechanical regulations of T cell differentiation are still unknown. Studying these questions may widen our understanding of T cell mechanobiology and improve our control of T cell responses.

Synthetic materials are still the most practical platform so far for studying T cell mechanobiology as their physicochemical properties can be easily adjusted. However, a better

understanding and control of the chemistry and materials used for studying T cell mechanical responses is necessary as reports so far show that T cells display different stiffness-dependent behaviors on different materials.[3,46,51] For example, T cell spreading on a PDMS elastomer substrate is drastically different from that on a PA hydrogel substrate despite exhibiting the same stiffness.[45] Paradoxically, increasing stiffness of HA hydrogel has been reported to suppress T cell activation[51] although increased stiffness induces stronger T cell activation on other materials (e.g., PA, PDMS, and alginate). These reports underline the urgent need to dissect some key parameters (e.g., chemical structure, hydrophobicity, and porosity) in material design, and unify these findings. Furthermore, in most in vitro studies so far, mechanical cues including stiffness and topography are static and lack the dynamic nature of the physiological microenvironment. Incorporating mechanical cues resembling the dynamics of biomechanics in tissues, such as viscoelasticity and stress-stiffening, will provide new insights into T cell mechanobiology. In general, any controllable system that can fully recapitulate the physicochemical properties of natural APCs will enhance our understanding of T cell mechanobiology during activation and potentially foster more precisely controlled mechanical immunoengineering strategies.

To better understand T cell mechanobiology, measuring and monitoring the spatiotemporal variation of forces applied on or exerted by T cells is essential. To date, there is a lack of technologies enabling in vivo monitoring of mechanical forces at the cellular level. Recently, Vorselen et al. developed a microparticle-based platform for traction force microscopy and applied it to T cell force measurement.[90] This microparticle-based platform, which can be injected in tissues using needles, shows promise as a method to record the force profile of single T cells in vivo. Directly measuring forces between T cells and target cells remains a significant challenge. To this end, DNA force probes hold special promise to measure such intercellular forces in vivo as it provides good sensitivity in the pN-range and quantitative readouts.[91]

Applying precise external forces to large scale T cell populations (thousands to millions and even more) remains challenging due to the complexity and low throughput of the current techniques, therefore curtailing studies on mechano-responses of T cells at population scale. Literature in mechanical engineering provides numerous examples of systems that could potentially be applied to T cells, including magnetic particles-based systems,[92] 2D stretching systems,[93] mechanical compression,[94] and microfluidic systems.[71] For example, using optical tweezer, Kim et al. claimed that a shear (tangential) force but not a normal force on CD3 could

trigger its downstream signaling.[29] To further confirm this results in a high-throughput manner, a microfluidic system with flow chamber could be used to apply shear stress to a large population of T cells. Overall, applying these methods for large-scale studies could potentially enable researchers to decipher the relationship between external forces and cellular phenotypes, metabolism, and effector functions.

Moving this highly interdisciplinary field forward in clinical application will require a concerted effort among physicists, chemists, material scientists, immunologists, and clinicians. The experience and know-how gained in conventional biochemical immunoengineering (examples including clinical trials NCT01753089 and NCT03815682 reported in clinicaltrials.gov) could potentially guide these translations. For example, armored CAR-T cells express supporting ligands or cytokines to improve their activity through genetic engineering. Mimicking armored CAR-T design, one can genetically modify T cells with a mechano-sensing circuit for mechano-controlled expression of CAR or supporting cytokines. Such a system has the potential to provide additional specificity targeting cancer when combined with biochemical immunoengineering. In addition, key therapeutic targets regarded solely as biochemical receptors, such as programmed cell death protein-1 (PD-1),[95] provide a cohort of important subjects for studying the potential role of mechanical cues in regulating their signaling and functions. For example, anti-PD-1 antibodies (aPD-1) have been used to block the PD-1/programmed death ligand 1/2 (PD-L1/2) interactions and reverse T cell exhaustion in tumor. Interestingly, a recent report shows that T cells exert stronger tensile tension along the PD-1/aPD-1 axis than that on the PD-1/PD-L2 axis,[96] implying that mechanical force may underlie the differential T cell responses upon binding to aPD-1 versus PD-L1/2. It is fundamentally interesting and therapeutically relevant to determine whether mechanical force plays a role in PD-1 signaling and thus regulating T cell exhaustion. Finally, as other immune cells, such as B cells and macrophages,[97,98] are also known to sense and respond to mechanical cues, we envision that the principles discussed here will potentially advance mechanical immunoengineering in other immune cell types.

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29

30 # References

31 (1) Basu, R.; Whitlock, B. M.; Husson, J.; Le Floc'h, A.; Jin, W.; Oyler-Yaniv, A.; Dotiwala, F.;
32 Giannone, G.; Hivroz, C.; Biais, N.; Lieberman, J.; Kam, L. C.; Huse, M. Cytotoxic T Cells Use
33 Mechanical Force to Potentiate Target Cell Killing. *Cell* **2016**, *165*, 100–110.

1 (2) Lambert, L. H.; Goebrecht, G. K. E.; De Leo, S. E.; O'Connor, R. S.; Nunez-Cruz, S.; Li, T. De;
2 Yuan, J.; Milone, M. C.; Kam, L. C. Improving T Cell Expansion with a Soft Touch. *Nano Lett.*
3 **2017**, *17*, 821–826.
4 (3) Majedi, F. S.; Hasani-Sadrabadi, M. M.; Thauland, T. J.; Li, S.; Bouchard, L.-S.; Butte, M. J. T-
5 Cell Activation Is Modulated by the 3D Mechanical Microenvironment. *Biomaterials* **2020**, *252*,
6 120058.
7 (4) Lei, K.; Li, T. T Cell Force-Responsive Delivery of Anticancer Drugs Using Mesoporous Silica
8 Microparticles. *Mater. Horizons* **2020**, doi:10.1039/D0MH01285H.
9 (5) June, C. H.; O'Connor, R. S.; Kawalekar, O. U.; Ghassemi, S.; Milone, M. C. CAR T Cell
10 Immunotherapy for Human Cancer. *Science* **2018**, *359*, 1361–1365.
11 (6) Barber, D. L.; Wherry, E. J.; Masopust, D.; Zhu, B.; Allison, J. P.; Sharpe, A. H.; Freeman, G.
12 J.; Ahmed, R. Restoring Function in Exhausted CD8 T Cells during Chronic Viral Infection.
13 *Nature* **2006**, *439*, 682–687.
14 (7) Bluestone, J. A.; Buckner, J. H.; Fitch, M.; Gitelman, S. E.; Gupta, S.; Hellerstein, M. K.; Herold,
15 K. C.; Lares, A.; Lee, M. R.; Li, K.; Liu, W.; Long, S. A.; Masiello, L. M.; Nguyen, V.; Putnam,
16 A. L.; Rieck, M.; Sayre, P. H.; Tang, Q. Type 1 Diabetes Immunotherapy Using Polyclonal
17 Regulatory T Cells. *Sci. Transl. Med.* **2015**, *7*, 315ra189.
18 (8) Wei, S. C.; Duffy, C. R.; Allison, J. P. Fundamental Mechanisms of Immune Checkpoint
19 Blockade Therapy. *Cancer Discov.* **2018**, *8*, 1069–1086.
20 (9) Ribas, A.; Wolchok, J. D. Cancer Immunotherapy Using Checkpoint Blockade. *Science* **2018**,
21 *359*, 1350–1355.
22 (10) Fesnak, A. D.; June, C. H.; Levine, B. L. Engineered T Cells: The Promise and Challenges of
23 Cancer Immunotherapy. *Nat. Rev. Cancer* **2016**, *16*, 566–581.
24 (11) Kerkar, S. P.; Muranski, P.; Kaiser, A.; Boni, A.; Sanchez-Perez, L.; Yu, Z.; Palmer, D. C.;
25 Reger, R. N.; Borman, Z. A.; Zhang, L.; Morgan, R. A.; Gattinoni, L.; Rosenberg, S. A.;
26 Trinchieri, G.; Restifo, N. P. Tumor-Specific CD8+ T Cells Expressing Interleukin-12 Eradicate
27 Established Cancers in Lymphodepleted Hosts. *Cancer Res.* **2010**, *70*, 6725–6734.
28 (12) Tang, L.; Zheng, Y.; Melo, M. B.; Mabardi, L.; Castano, A. P.; Xie, Y.-Q.; Li, N.; Kudchodkar,
29 S. B.; Wong, H. C.; Jeng, E. K.; Maus, M. V; Irvine, D. J. Enhancing T Cell Therapy through
30 TCR-Signaling-Responsive Nanoparticle Drug Delivery. *Nat. Biotechnol.* **2018**, *36*, 707–716.
31 (13) Xie, Y. Q.; Arik, H.; Wei, L.; Zheng, Y.; Suh, H.; Irvine, D. J.; Tang, L. Redox-Responsive
32 Interleukin-2 Nanogel Specifically and Safely Promotes the Proliferation and Memory Precursor
33 Differentiation of Tumor-Reactive T-Cells. *Biomater. Sci.* **2019**, *7*, 1345–1357.
34 (14) Walling, B. L.; Kim, M. LFA-1 in T Cell Migration and Differentiation. *Front. Immunol.* **2018**,
35 *9*, 952.
36 (15) Guimarães, C. F.; Gasperini, L.; Marques, A. P.; Reis, R. L. The Stiffness of Living Tissues and

1 Its Implications for Tissue Engineering. *Nat. Rev. Mater.* **2020**, *5*, 351–370.
2 (16) Hu, K. H.; Butte, M. J. T Cell Activation Requires Force Generation. *J. Cell Biol.* **2016**, *213*,
3 535–542.
4 (17) Blumenthal, D.; Avery, L.; Chandra, V.; Burkhardt, J. K. Mouse T-Cell Priming Is Enhanced
5 by Maturation-Dependent Stiffening of the Dendritic Cell Cortex. *bioRxiv* **2020**, doi:
6 10.1101/680132.
7 (18) Comrie, W. A.; Burkhardt, J. K. Action and Traction: Cytoskeletal Control of Receptor
8 Triggering at the Immunological Synapse. *Front. Immunol.* **2016**, *7*, 68.
9 (19) Huse, M. Mechanical Forces in the Immune System. *Nat. Rev. Immunol.* **2017**, *17*, 679–690.
10 (20) Zhu, C.; Chen, W.; Lou, J.; Rittase, W.; Li, K. Mechanosensing through Immunoreceptors. *Nat.*
11 *Immunol.* **2019**, *10*, 1269–1278.
12 (21) Harrison, D. L.; Fang, Y.; Huang, J. T-Cell Mechanobiology: Force Sensation, Potentiation, and
13 Translation. *Front. Phys.* **2019**, *7*, 45.
14 (22) Fritzsche, M.; Dustin, M. L. *Organization of Immunological Synapses and Kinapses*; Putterman,
15 C., Cowburn, D., Almo, S., Eds.; Elsevier Inc.: London, 2018.
16 (23) Saxman, K.; Ammer, D. H. The Reaction-Limited Kinetics of Membrane-to-Surface Adhesion
17 and Detachment. *Proc. R. Soc. Lond. B* **1988**, *234*, 55–83.
18 (24) Thomas, W. E.; Trintchina, E.; Forero, M.; Vogel, V.; Sokurenko, E. V. Bacterial Adhesion to
19 Target Cells Enhanced by Shear Force. *Cell* **2002**, *109*, 913–923.
20 (25) Marshall, B. T.; Long, M.; Piper, J. W.; Yago, T.; McEver, R. P.; Zhu, C. Direct Observation of
21 Catch Bonds Involving Cell-Adhesion Molecules. *Nature* **2003**, *423*, 190–193.
22 (26) Liu, B.; Chen, W.; Evavold, B. D.; Zhu, C. Accumulation of Dynamic Catch Bonds between
23 TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell* **2014**, *157*, 357–368.
24 (27) Hong, J.; Ge, C.; Jothikumar, P.; Yuan, Z.; Liu, B.; Bai, K.; Li, K.; Rittase, W.; Shinzawa, M.;
25 Zhang, Y.; Palin, A.; Love, P.; Yu, X.; Salaita, K.; Evavold, B. D.; Singer, A.; Zhu, C. A TCR
26 Mechanotransduction Signaling Loop Induces Negative Selection in the Thymus. *Nat. Immunol.*
27 **2018**, *19*, 1379–1390.
28 (28) Li, Y. C.; Chen, B. M.; Wu, P. C.; Cheng, T. L.; Kao, L. S.; Tao, M. H.; Lieber, A.; Roffler, S.
29 R. Mechanical Forces Acting on T Cells Immobilized via the TCR Complex Can Trigger TCR
30 Signaling. *J. Immunol.* **2010**, *184*, 5959–5963.
31 (29) Kim, S. T.; Takeuchi, K.; Sun, Z. Y. J.; Touma, M.; Castro, C. E.; Fahmy, A.; Lang, M. J.;
32 Wagner, G.; Reinherz, E. L. The αβ T Cell Receptor Is an Anisotropic Mechanosensor. *J. Biol.*
33 *Chem.* **2009**, *284*, 31028–31037.
34 (30) Monks, C. R. F.; Freiberg, B. A.; Kupfer, H.; Sciaky, N.; Kupfer, A. Three-Dimensional
35 Segregation of Supramolecular Activation Clusters in T Cells. *Nature* **1998**, *395*, 82–86.
36 (31) Grakoui, A.; Bromley, S. K.; Sumen, C.; Davis, M. M.; Shaw, A. S.; Allen, P. M.; Dustin, M.

1 L. The Immunological Synapse: A Molecular Machine Controlling T Cell Activation. *Science*
2 **1999**, *285*, 221–227.
3 (32) Comrie, W. A.; Babich, A.; Burkhardt, J. K. F-Actin Flow Drives Affinity Maturation and
4 Spatial Organization of LFA-1 at the Immunological Synapse. *J. Cell Biol.* **2015**, *208*, 475–491.
5 (33) Husson, J.; Chemin, K.; Bohineust, A.; Hivroz, C.; Henry, N. Force Generation upon T Cell
6 Receptor Engagement. *PLoS One* **2011**, *6*, e19680.
7 (34) Bashoura, K. T.; Gondarenko, A.; Chen, H.; Shen, K.; Liu, X.; Huse, M.; Hone, J. C.; Kam, L.
8 C. CD28 and CD3 Have Complementary Roles in T-Cell Traction Forces. *Proc. Natl. Acad. Sci.*
9 *U. S. A.* **2014**, *111*, 2241–2246.
10 (35) Hui, K. L.; Balagopalan, L.; Samelson, L. E.; Upadhyaya, A. Cytoskeletal Forces during
11 Signaling Activation in Jurkat T-Cells. *Mol. Biol. Cell* **2015**, *26*, 685–695.
12 (36) Liu, Y.; Blanchfield, L.; Ma, V. P.-Y.; Andargachew, R.; Galior, K.; Liu, Z.; Evavold, B.; Salaita,
13 K. DNA-Based Nanoparticle Tension Sensors Reveal That T-Cell Receptors Transmit Defined
14 PN Forces to Their Antigens for Enhanced Fidelity. *Proc. Natl. Acad. Sci.* **2016**, *113* (20), 5610–
15 5615.
16 (37) Judokusumo, E.; Tabdanov, E.; Kumari, S.; Dustin, M. L.; Kam, L. C. Mechanosensing in T
17 Lymphocyte Activation. *Biophys. J.* **2012**, *102*, L5–L7.
18 (38) Tamzalit, F.; Wang, M. S.; Jin, W.; Tello-Lafoz, M.; Boyko, V.; Heddleston, J. M.; Black, C.
19 T.; Kam, L. C.; Huse, M. Interfacial Actin Protrusions Mechanically Enhance Killing by
20 Cytotoxic T Cells. *Sci. Immunol.* **2019**, *4*, eaav5445.
21 (39) Anselme, K.; Bigerelle, M. Role of Materials Surface Topography on Mammalian Cell
22 Response. *Int. Mater. Rev.* **2011**, *56*, 243–266.
23 (40) Nemir, S.; West, J. L. Synthetic Materials in the Study of Cell Response to Substrate Rigidity.
24 *Ann. Biomed. Eng.* **2010**, *38*, 2–20.
25 (41) Meng, K. P.; Majedi, F. S.; Thauland, T. J.; Butte, M. J. Mechanosensing through YAP Controls
26 T Cell Activation and Metabolism. *J. Exp. Med.* **2020**, *217*, e20200053.
27 (42) Levental, K. R.; Yu, H.; Kass, L.; Lakins, J. N.; Egeblad, M.; Erler, J. T.; Fong, S. F. T.; Csiszar,
28 K.; Giaccia, A.; Weninger, W.; Yamauchi, M.; Gasser, D. L.; Weaver, V. M. Matrix
29 Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling. *Cell* **2009**, *139*, 891–
30 906.
31 (43) Schraml, B. U.; Reis e Sousa, C. Defining Dendritic Cells. *Curr. Opin. Immunol.* **2015**, *32*, 13–
32 20.
33 (44) O'Connor, R. S.; Hao, X.; Shen, K.; Bashour, K.; Akimova, T.; Hancock, W. W.; Kam, L. C.;
34 Milone, M. C. Substrate Rigidity Regulates Human T Cell Activation and Proliferation. *J.*
35 *Immunol.* **2012**, *189*, 1330–1339.
36 (45) Wahl, A.; Dinet, C.; Dillard, P.; Nassereddine, A.; Puech, P. H.; Limozin, L.; Sengupta, K.

Biphasic Mechanosensitivity of T Cell Receptor-Mediated Spreading of Lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 5908–5913.

(46) Saitakis, M.; Dogniaux, S.; Goudot, C.; Bufi, N.; Asnacios, S.; Maurin, M.; Randriamampita, C.; Asnacios, A.; Hivroz, C. Different TCR-Induced T Lymphocyte Responses Are Potentiated by Stiffness with Variable Sensitivity. *Elife* **2017**, *6*, e23190.

(47) Azadi, S.; Tafazzoli-Shadpour, M.; Soleimani, M.; Warkiani, M. E. Modulating Cancer Cell Mechanics and Actin Cytoskeleton Structure by Chemical and Mechanical Stimulations. *J. Biomed. Mater. Res. - Part A* **2019**, *107A*, 1569–1581.

(48) Tello-lafoz, M.; Srpan, K.; Hu, J.; Romin, Y.; Calò, A.; Katharine, C. Cytotoxic Lymphocytes Use Mechanosurveillance to Target Biophysical Vulnerabilities in Cancer. *bioRxiv* **2020**, doi: 10.1101/2020.04.21.054304.

(49) Nataraj, N. M.; Dang, A. P.; Kam, L. C.; Lee, J. H. Ex Vivo Induction of Regulatory T Cells from Conventional CD4+ T Cells Is Sensitive to Substrate Rigidity. *J. Biomed. Mater. Res. - Part A* **2018**, *106A*, 3001–3008.

(50) Romano, M.; Fanelli, G.; Albany, C. J.; Giganti, G.; Lombardi, G. Past, Present, and Future of Regulatory T Cell Therapy in Transplantation and Autoimmunity. *Front. Immunol.* **2019**, *10*, 43.

(51) Hickey, J. W.; Dong, Y.; Chung, J. W.; Salathe, S. F.; Pruitt, H. C.; Li, X.; Chang, C.; Fraser, A. K.; Bessell, C. A.; Ewald, A. J.; Gerecht, S.; Mao, H. Q.; Schneck, J. P. Engineering an Artificial T-Cell Stimulating Matrix for Immunotherapy. *Adv. Mater.* **2019**, *31*, 1807359.

(52) Kim, Y.; Kumar, S. CD44-Mediated Adhesion to Hyaluronic Acid Contributes to Mechanosensing and Invasive Motility. *Mol. Cancer Res.* **2014**, *12*, 1416–1429.

(53) Panciera, T.; Citron, A.; Di Biagio, D.; Battilana, G.; Gandin, A.; Giulitti, S.; Forcato, M.; Bicciato, S.; Panzetta, V.; Fusco, S.; Azzolin, L.; Totaro, A.; Dei Tos, A. P.; Fassan, M.; Vindigni, V.; Bassetto, F.; Rosato, A.; Brusatin, G.; Cordenonsi, M.; Piccolo, S. Reprogramming Normal Cells into Tumour Precursors Requires ECM Stiffness and Oncogene-Mediated Changes of Cell Mechanical Properties. *Nat. Mater.* **2020**, *19*, 797–806.

(54) Yamakita, Y.; Matsumura, F.; Lipscomb, M. W.; Chou, P.; Werlen, G.; Burkhardt, J. K.; Yamashiro, S. Fascin1 Promotes Cell Migration of Mature Dendritic Cells. *J. Immunol.* **2011**, *186*, 2850–2859.

(55) Fisher, P. J.; Bulur, P. A.; Vuk-Pavlovic, S.; Prendergast, F. G.; Dietz, A. B. Dendritic Cell Microvilli: A Novel Membrane Structure Associated with the Multifocal Synapse and T-Cell Clustering. *Blood* **2008**, *112*, 5037–5045.

(56) Sunshine, J. C.; Perica, K.; Schneck, J. P.; Green, J. J. Particle Shape Dependence of CD8+ T Cell Activation by Artificial Antigen Presenting Cells. *Biomaterials* **2014**, *35*, 269–277.

(57) Meyer, R. A.; Sunshine, J. C.; Perica, K.; Kosmides, A. K.; Aje, K.; Schneck, J. P.; Green, J. J.

Biodegradable Nanoellipsoidal Artificial Antigen Presenting Cells for Antigen Specific T-Cell Activation. *Small* **2015**, *11*, 1519–1525.

(58) Cochran, J. R.; Cameron, T. O.; Stone, J. D.; Lubetsky, J. B.; Stern, L. J. Receptor Proximity, Not Intermolecular Orientation, Is Critical Tor Triggering T-Cell Activation. *J. Biol. Chem.* **2001**, *276*, 28068–28074.

(59) Delcassian, D.; Depoil, D.; Rudnicka, D.; Liu, M.; Davis, D. M.; Dustin, M. L.; Dunlop, I. E. Nanoscale Ligand Spacing Influences Receptor Triggering in T Cells and NK Cells. *Nano Lett.* **2013**, *13*, 5608–5614.

(60) Cai, H.; Muller, J.; Depoil, D.; Mayya, V.; Sheetz, M. P.; Dustin, M. L.; Wind, S. J. Full Control of Ligand Positioning Reveals Spatial Thresholds for T Cell Receptor Triggering. *Nat. Nanotechnol.* **2018**, *13*, 610–617.

(61) Matic, J.; Deeg, J.; Scheffold, A.; Goldstein, I.; Spatz, J. P. Fine Tuning and Efficient T Cell Activation with Stimulatory aCD3 Nanoarrays. *Nano Lett.* **2013**, *13*, 5090–5097.

(62) Guasch, J.; Hoffmann, M.; Diemer, J.; Riahinezhad, H.; Neubauer, S.; Kessler, H.; Spatz, J. P. Combining Adhesive Nanostructured Surfaces and Costimulatory Signals to Increase T Cell Activation. *Nano Lett.* **2018**, *18*, 5899–5904.

(63) Deeg, J.; Axmann, M.; Matic, J.; Liapis, A.; Depoil, D.; Afrose, J.; Curado, S.; Dustin, M. L.; Spatz, J. P. T Cell Activation Is Determined by the Number of Presented Antigens. *Nano Lett.* **2013**, *13*, 5619–5626.

(64) Friedl, P.; Den Boer, A. T.; Gunzer, M. Tuning Immune Responses: Diversity and Adaptation of the Immunological Synapse. *Nat. Rev. Immunol.* **2005**, *5*, 532–545.

(65) Mossman, K. D.; Campi, G.; Groves, J. T.; Dustin, M. L. Altered TCR Signaling from Geometrically Repatterned Immunological Synapses. *Science* **2005**, *310*, 1191–1193.

(66) Čemerski, S.; Das, J.; Giurisato, E.; Markiewicz, M. A.; Allen, P. M.; Chakraborty, A. K.; Shaw, A. S. The Balance between T Cell Receptor Signaling and Degradation at the Center of the Immunological Synapse Is Determined by Antigen Quality. *Immunity* **2008**, *29*, 414–422.

(67) Doh, J.; Irvine, D. J. Immunological Synapse Arrays: Patterned Protein Surfaces That Modulate Immunological Synapse Structure Formation in T Cells. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 5700–5705.

(68) Yokosuka, T.; Kobayashi, W.; Sakata-Sogawa, K.; Takamatsu, M.; Hashimoto-Tane, A.; Dustin, M. L.; Tokunaga, M.; Saito, T. Spatiotemporal Regulation of T Cell Costimulation by TCR-CD28 Microclusters and Protein Kinase C θ Translocation. *Immunity* **2008**, *29*, 589–601.

(69) Shen, K.; Thomas, V. K.; Dustin, M. L.; Kam, L. C. Micropatterning of Costimulatory Ligands Enhances CD4+ T Cell Function. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7791–7796.

(70) Bashour, K. T.; Tsai, J.; Shen, K.; Lee, J.-H.; Sun, E.; Milone, M. C.; Dustin, M. L.; Kam, L. C. Cross Talk between CD3 and CD28 Is Spatially Modulated by Protein Lateral Mobility. *Mol.*

Cell. Biol. **2014**, *34*, 955–964.
(71) Lee, J. H.; Dustin, M. L.; Kam, L. C. A Microfluidic Platform Reveals Differential Response of Regulatory T Cells to Micropatterned Costimulation Arrays. *Integr. Biol.* **2015**, *7*, 1442–1453.
(72) Min, S.; Jeon, Y. S.; Jung, H. J.; Khatua, C.; Li, N.; Bae, G.; Choi, H.; Hong, H.; Shin, J. E.; Ko, M. J.; Ko, H. S.; Jun, I.; Fu, H. E.; Kim, S. H.; Thangam, R.; Song, J. J.; Dravid, V. P.; Kim, Y. K.; Kang, H. Independent Tuning of Nano-Ligand Frequency and Sequences Regulates the Adhesion and Differentiation of Stem Cells. *Adv. Mater.* **2020**, *32*, 2004300.
(73) Sage, P. T.; Varghese, L. M.; Martinelli, R.; Sciuto, T. E.; Kamei, M.; Dvorak, A. M.; Springer, T. A.; Sharpe, A. H.; Carman, C. V. Antigen Recognition Is Facilitated by Invadosome-like Protrusions Formed by Memory/Effector T Cells. *J. Immunol.* **2012**, *188*, 3686–3699.
(74) Rossy, J.; Laufer, J. M.; Legler, D. F. Role of Mechanotransduction and Tension in t Cell Function. *Front. Immunol.* **2018**, *9*, 2638.
(75) Stanley, P.; Smith, A.; McDowall, A.; Nicol, A.; Zicha, D.; Hogg, N. Intermediate-Affinity LFA-1 Binds α-Actinin-1 to Control Migration at the Leading Edge of the T Cell. *EMBO J.* **2008**, *27*, 62–75.
(76) Leslie, N. R.; Downes, C. P. PTEN Function: How Normal Cells Control It and Tumour Cells Lose It. *Biochem. J.* **2004**, *382*, 1–11.
(77) Benjamin Mathias Whitlock. Enhancing Cytotoxic T Cell Killing by PTEN Depletion, Ph.D. Thesis, Cornell University, 2018.
(78) Hui, K. L.; Upadhyaya, A. Dynamic Microtubules Regulate Cellular Contractility during T-Cell Activation. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E4175–E4183.
(79) Tabdanov, E.; Rodríguez-Merced, N.; Cartagena-Rivera, A.; Puram, V.; Callaway, M.; Ensminger, E.; Pomeroy, E.; Yamamoto, K.; Lahr, W.; Webber, B.; Moriarity, B.; Zhovmer, A.; Provenzano, P. Engineering T Cells to Enhance 3D Migration through Structurally and Mechanically Complex Tumor Microenvironments. *bioRxiv* **2020**, doi: 10.1101/2020.04.21.051615.
(80) Tamzalit, F.; Tran, D.; Jin, W.; Boyko, V.; Bazzi, H.; Kepecs, A.; Kam, L. C.; Anderson, K. V.; Huse, M. Centrioles Control the Capacity, but Not the Specificity, of Cytotoxic T Cell Killing. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 4310–4319.
(81) de la Zerda, A.; Kratochvil, M. J.; Suhar, N. A.; Heilshorn, S. C. Review: Bioengineering Strategies to Probe T Cell Mechanobiology. *APL Bioeng.* **2018**, *2*, 021501.
(82) Krieg, M.; Fläschner, G.; Alsteens, D.; Gaub, B. M.; Roos, W. H.; Wuite, G. J. L.; Gaub, H. E.; Gerber, C.; Dufrêne, Y. F.; Müller, D. J. Atomic Force Microscopy-Based Mechanobiology. *Nat. Rev. Phys.* **2019**, *1*, 41–57.
(83) Perica, K.; Schneck, J. Magnetic Field-Induced Receptor Clustering By Nanoparticles Stimulates Anti-Tumor Activity In Vivo. *ACS Nano* **2014**, *8*, 2252–2260.

(84) Majedi, F. S.; Hasani-Sadrabadi, M. M.; Thauland, T. J.; Li, S.; Bouchard, L. S.; Butte, M. J. Augmentation of T-Cell Activation by Oscillatory Forces and Engineered Antigen-Presenting Cells. *Nano Lett.* **2019**, *19*, 6945–6954.

(85) Liu, Z.; Liu, Y.; Chang, Y.; Seyf, H. R.; Henry, A.; Mattheyses, A. L.; Yehl, K.; Zhang, Y.; Huang, Z.; Salaita, K. Nanoscale Optomechanical Actuators for Controlling Mechanotransduction in Living Cells. *Nat. Methods* **2016**, *13*, 143–146.

(86) Douplik, A.; Saiko, G.; Schelkanova, I.; Tuchin, V. V. *The Response of Tissue to Laser Light*; Jelínková, H., Ed.; Woodhead Publishing Limited: Cambridge, UK, 2013.

(87) Carovac, A.; Smajlovic, F.; Junuzovic, D. Application of Ultrasound in Medicine. *Acta Inform. Medica* **2011**, *19*, 168–171.

(88) Pan, Y.; Yoon, S.; Sun, J.; Huang, Z.; Lee, C.; Allen, M.; Wu, Y.; Chang, Y. J.; Sadelain, M.; Kirk Shung, K.; Chien, S.; Wang, Y. Mechanogenetics for the Remote and Noninvasive Control of Cancer Immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 992–997.

(89) Crompton, J. G.; Sukumar, M.; Restifo, N. P. Uncoupling T-cell Expansion from Effector Differentiation in Cell-based Immunotherapy. *Immunol. Rev.* **2014**, *257*, 264–276.

(90) Vorselen, D.; Wang, Y.; de Jesus, M. M.; Shah, P. K.; Footer, M. J.; Huse, M.; Cai, W.; Theriot, J. A. Microparticle Traction Force Microscopy Reveals Subcellular Force Exertion Patterns in Immune Cell–Target Interactions. *Nat. Commun.* **2020**, *11*, 20.

(91) Zhao, B.; Li, N.; Xie, T.; Bagheri, Y.; Liang, C.; Keshri, P.; Sun, Y.; You, M. Quantifying Tensile Forces at Cell-Cell Junctions with a DNA-Based Fluorescent Probe. *Chem. Sci.* **2020**, *11*, 8558–8566.

(92) Tseng, P.; Judy, J. W.; Di Carlo, D. Magnetic Nanoparticle-Mediated Massively Parallel Mechanical Modulation of Single-Cell Behavior. *Nat. Methods* **2012**, *9*, 1113–1119.

(93) Kamble, H.; Barton, M. J.; Jun, M.; Park, S.; Nguyen, N. T. Cell Stretching Devices as Research Tools: Engineering and Biological Considerations. *Lab Chip* **2016**, *16*, 3193–3203.

(94) Solis, A. G.; Bielecki, P.; Steach, H. R.; Sharma, L.; Harman, C. C. D.; Yun, S.; de Zoete, M. R.; Warnock, J. N.; To, S. D. F.; York, A. G.; Mack, M.; Schwartz, M. A.; Dela Cruz, C. S.; Palm, N. W.; Jackson, R.; Flavell, R. A. Mechanosensation of Cyclical Force by PIEZO1 Is Essential for Innate Immunity. *Nature* **2019**, *573*, 69–74.

(95) Zhao, Y.; Lee, C. K.; Lin, C. H.; Gassen, R. B.; Xu, X.; Huang, Z.; Xiao, C.; Bonorino, C.; Lu, L. F.; Bui, J. D.; Hui, E. PD-L1:CD80 Cis-Heterodimer Triggers the Co-Stimulatory Receptor CD28 While Repressing the Inhibitory PD-1 and CTLA-4 Pathways. *Immunity* **2019**, *51*, 1059-1073.e9.

(96) Ma, R.; Kellner, A. V.; Ma, V. P. Y.; Su, H.; Deal, B. R.; Brockman, J. M.; Salaita, K. DNA Probes That Store Mechanical Information Reveal Transient Piconewton Forces Applied by T Cells. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 16949–16954.

(97) Molloy, J. E.; Tolar, P. B Cells Use Mechanical Energy to Discriminate Antigen Affinities. *Science* **2013**, *340*, 1587–1590.
(98) Jain, N.; Moeller, J.; Vogel, V. Mechanobiology of Macrophages: How Physical Factors Coregulate Macrophage Plasticity and Phagocytosis. *Annu. Rev. Biomed. Eng.* **2019**, *21*, 267–297.

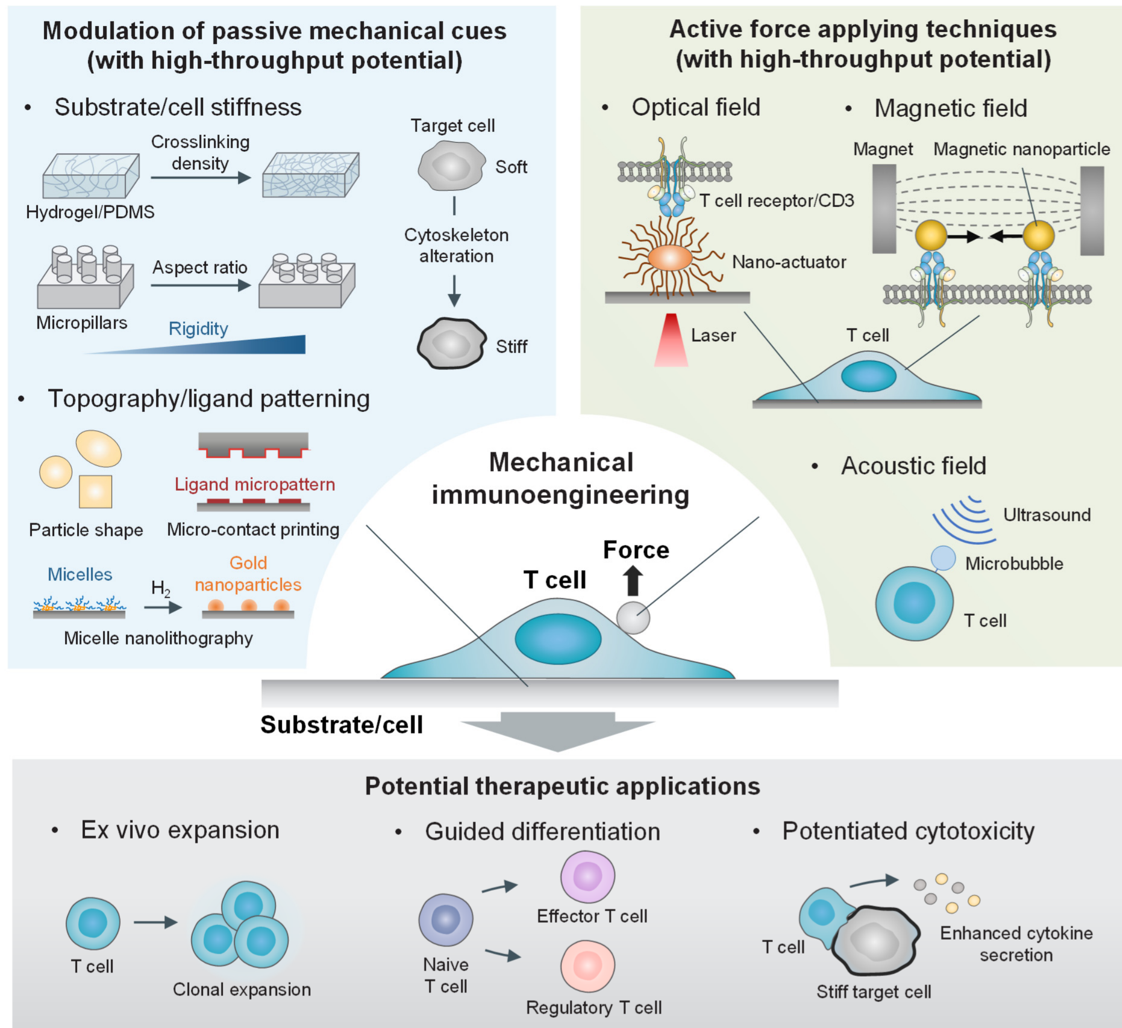
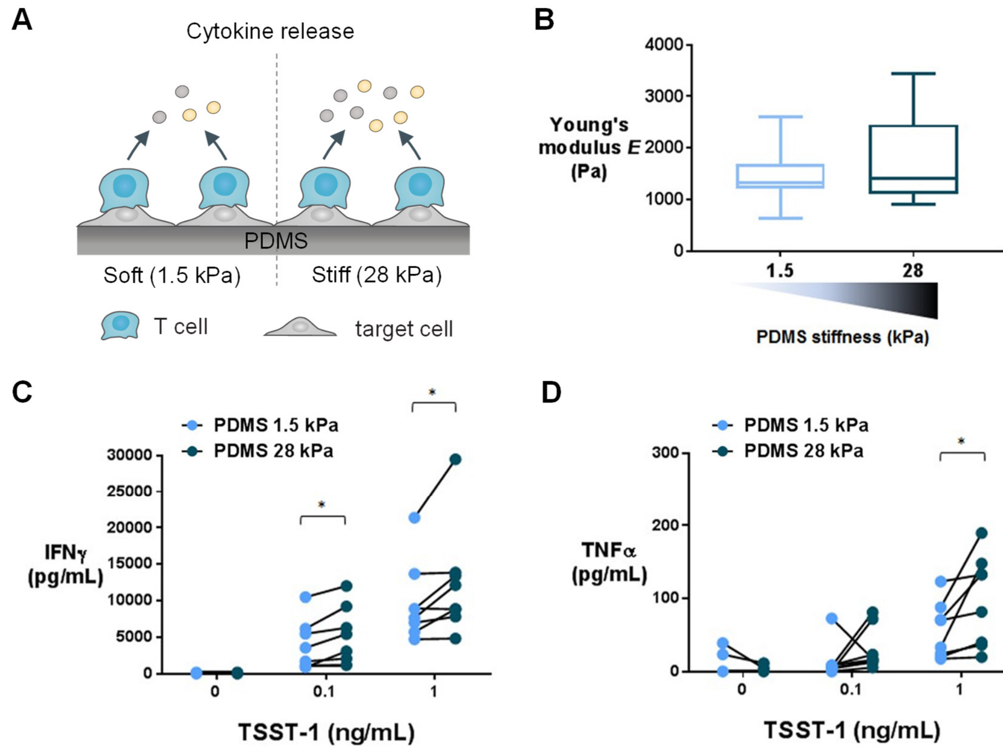


Figure 1. Schematic illustration of mechanical immunoengineering of T cells using passive mechanical cues or active mechanical forces for therapeutic applications. PDMS, polydimethylsiloxane.



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2 **Figure 2**. (A) Schematic of enhanced T cell polyfunctionality toward target cells on stiffer
3 substrate. (B) Young's modulus of HeLa-CIITA cells (HeLa cancer cells expressing MHC
4 class II molecules) cultured on PDMS substrates of different stiffness. (C, D) Production of
5 IFNγ (C) and TNFα (D) by human $CD4^+$ T cells interacting with HeLa-CIITA cells on PDMS
6 of varying stiffness in the presence of TSST-1 superantigen of different concentrations. PDMS,
7 polydimethylsiloxane. Adapted with permission from ref. 46. Copyright 2017 eLife Sciences
8 Publications.

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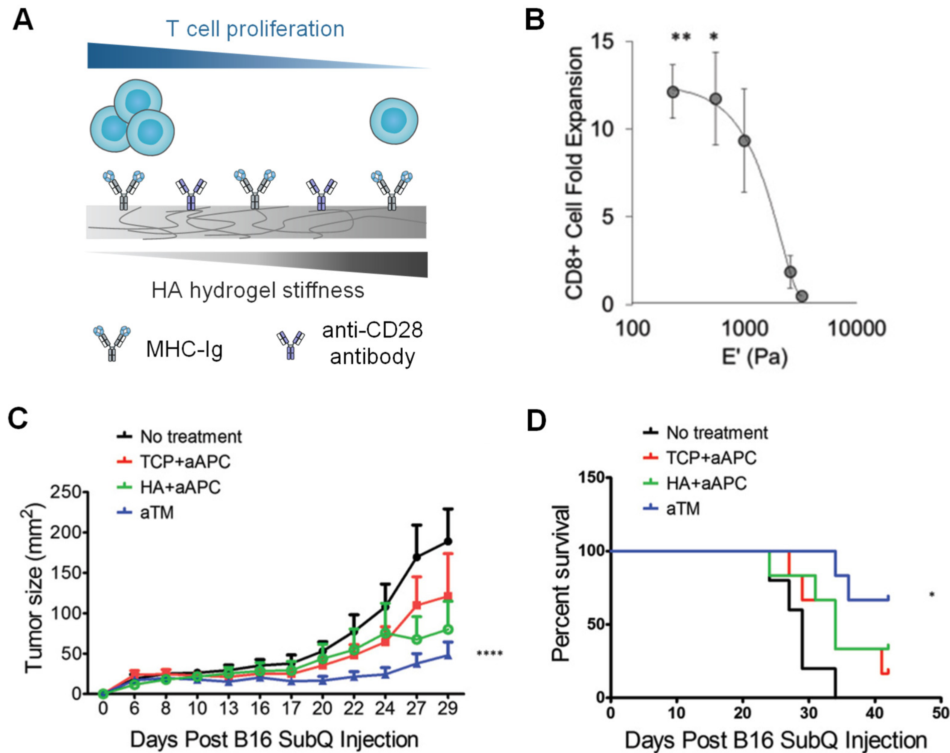

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2 **Figure 3**. (A) Schematic of stiffness-dependent T cell expansion on an artificial T cell
3 stimulating matrix (aTM) made from conjugating T cell stimulation signals to a hyaluronic
4 acid (HA) hydrogel. (B) Fold expansion of naïve $CD8^+$ T cells on aTMs with varying elastic
5 modulus (E′). (C, D) B16 tumor growth (C) and survival rate of mice (D) receiving adoptive
6 transferred T cells expanded by different methods. MHC-Ig, major histocompatibility
7 complex-immunoglobulin dimer; aAPC, artificial antigen-presenting cell; TCP, traditional
8 tissue culture plate; SubQ, subcutaneous. Adapted with permission from ref. 51. Copyright
9 2019 Wiley-VCH.

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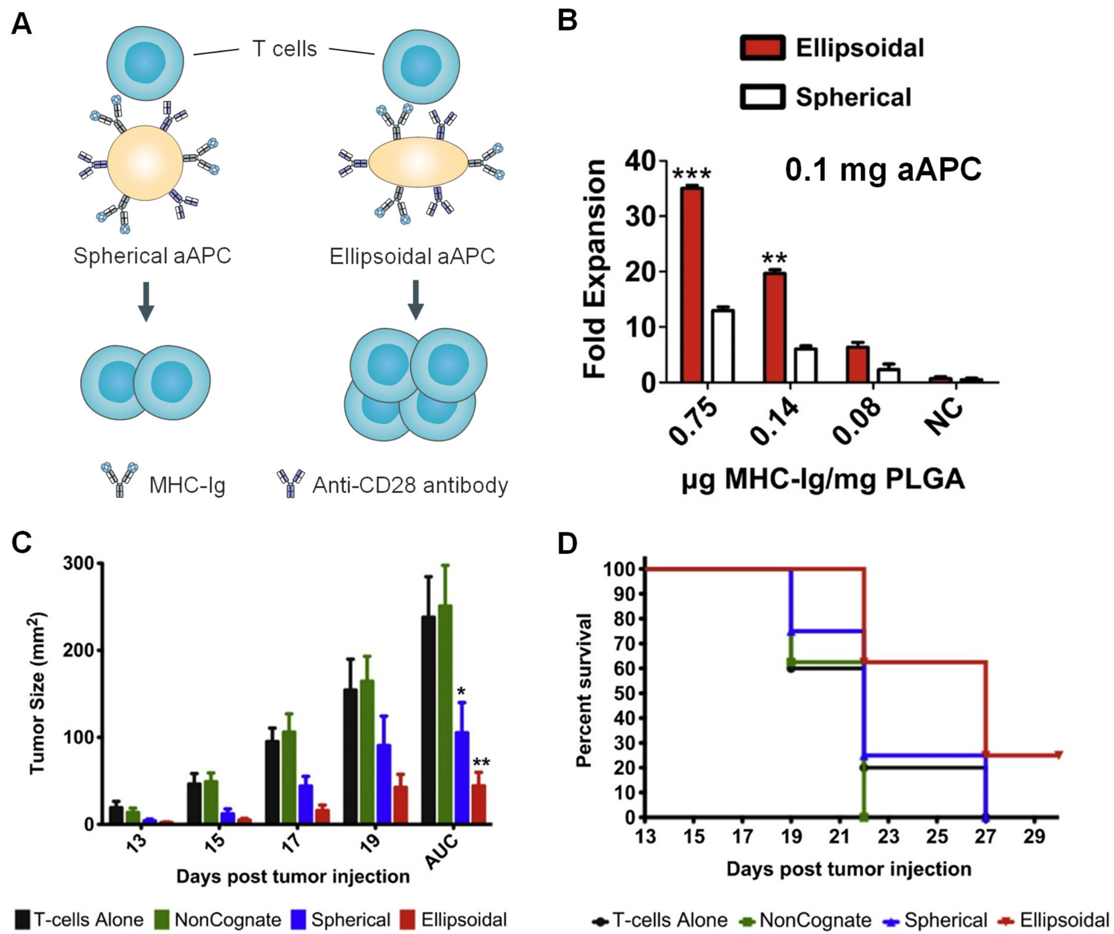
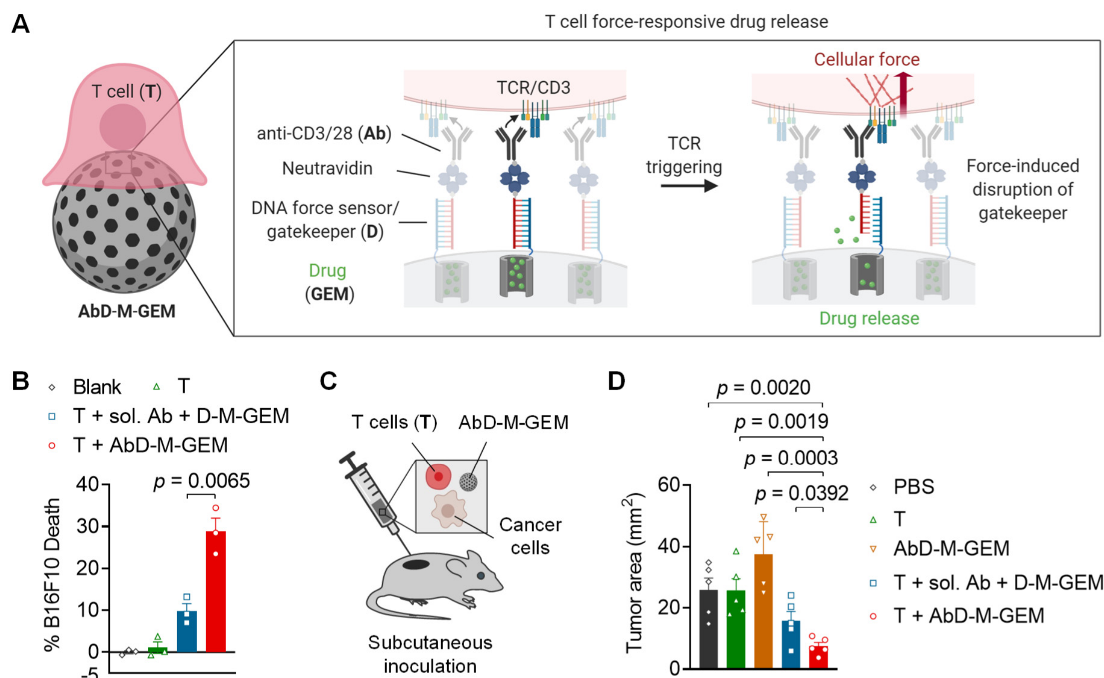


Figure 4. (A) Schematic of enhanced proliferation of T cells stimulated by an ellipsoidal artificial antigen-presenting cell (aAPC) compared to its spherical counterpart of similar volume (volume-weighted diameter ~6.7 µm). (B) Fold expansion of CD8^{+} T cells. (C, D) Tumor size (C) and survival rate (D) of B16 melanoma-bearing mice treated with naïve Pmel CD8^{+} T cells alone or combined with non-cognate ellipsoidal (NonCognate), cognate spherical, and cognate ellipsoidal aAPC. MHC-Ig, major histocompatibility complex-immunoglobulin dimer; PLGA, poly(lactic-*co*-glycolic acid); AUC, area under the curve. Adapted with permission from ref. 56. Copyright 2013 Elsevier.



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2 **Figure 5**. (A) Schematic of T cell (**T**) force-responsive drug release system based on a
3 mesoporous silica microparticle (**M**) capped with DNA force sensors (**D**). Such system (noted
4 as **AbD-M-GEM**) was loaded with an anticancer drug, gemcitabine (**GEM**). TCR, T cell
5 receptor; **Ab**, anti-CD3 and anti-CD28 antibodies. (B) Percentage of B16F10 cell death upon
6 the indicated treatments in vitro. (C) Schematic of an in vivo cancer prevention assay using T
7 cell force-responsive drug release system. (D) Average tumor areas at day 7 post inoculation
8 of MC38 cancer cells mixed with indicated agents. sol. Ab, soluble anti-CD3 and anti-CD28
9 antibodies. Adapted with permission from ref. 4. Copyright 2020 Royal Society of Chemistry.

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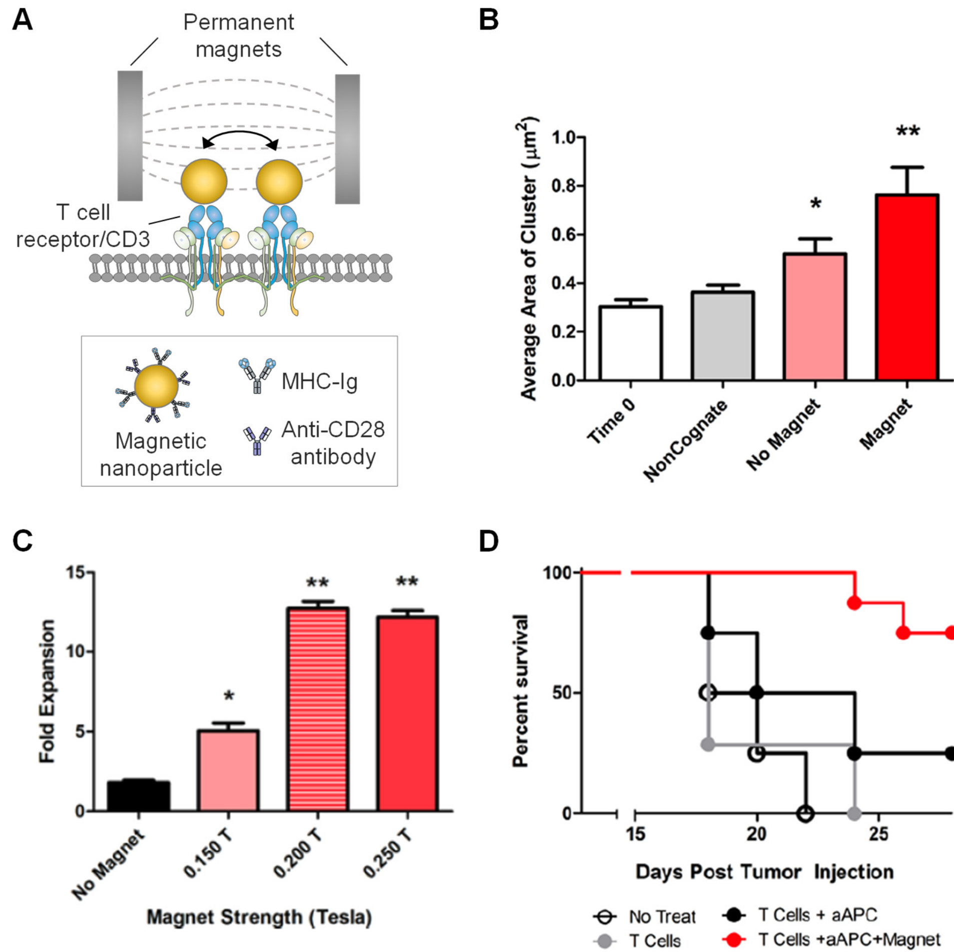

Figure 6. (A) Schematic of a magnetic nanoparticle-based system as an artificial antigen-presenting cell (aAPC) through induced T cell receptor (TCR)/CD3 clustering. (B, C) Average area of TCR/CD3 clusters (B) and fold expansion of T cells (C) in the presence of magnetic field. (D) Survival rate of mice bearing subcutaneous B16 tumors and receiving adoptive transfer of T cells activated by aAPC ex vivo in the presence of a magnetic field. MHC-Ig, major histocompatibility complex-immunoglobulin dimer. Adapted with permission from ref. 83. Copyright 2014 American Chemical Society.

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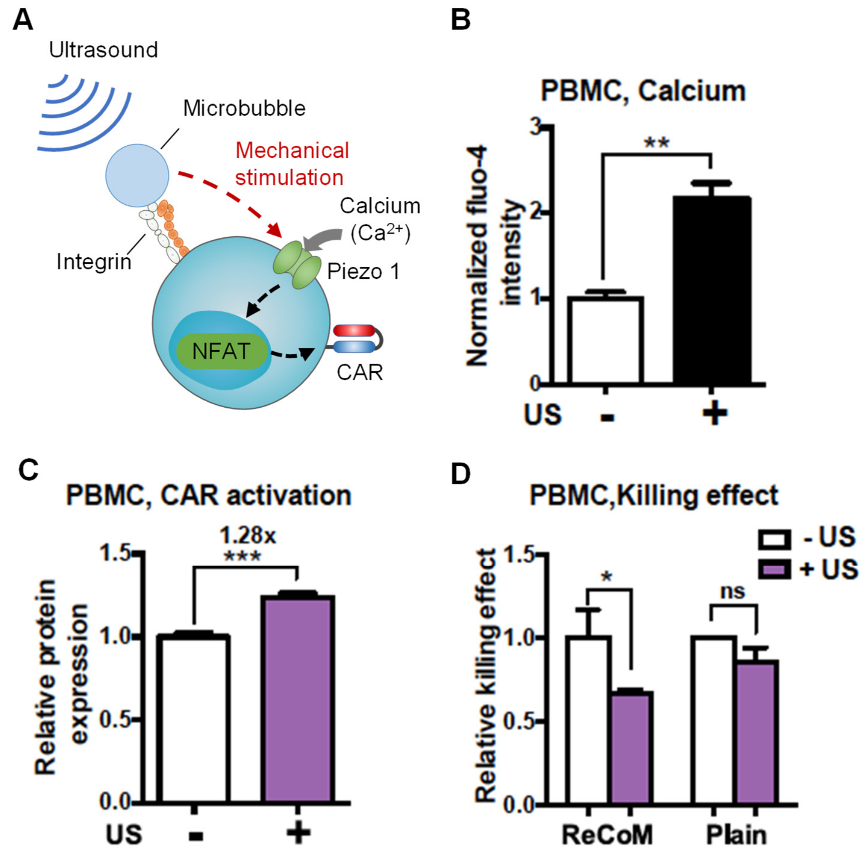

2 **Figure 7**. (A) Schematic of a remotely-controlled mechanogenetic (ReCoM) system for
3 ultrasound (US)-responsive activation of chimeric antigen receptor (CAR)-T cells. (B, C)
4 Calcium influx (B) and CAR expression (C) of engineered human CAR-T cells from human
5 PBMCs with or without US stimulation. (D) Relative viability of target tumor cells (Nalm6)
6 following co-incubation with CAR-T cells engineered with ReCoM system or native CAR-T
7 cells (plain). PBMCs, peripheral blood mononuclear cells. Adapted with permission from ref.
8 88. Copyright 2018 National Academy of Sciences USA.