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Review

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Mechanoimmunomodulation-based strategy on advancing tissue-engineered nanotopographic structures

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Abstract

The application of nanotopographic structures is considered a promising strategy for improving outcomes in tissue engineering. Nanotopographic structures-mediated immune responses have a more profound influence than the direct modulation of functional cell responses. However, the reported immunomodulatory effects of different nanotopographic structures are inconsistent and unpredictable. Therefore, it is necessary to further understand the general or fundamental biological mechanisms underlying nanotopographic structures-mediated immune regulation to fabricate structures with the desired immunomodulatory properties. Compared to the effects on protein absorption and physiochemical signals, the mechanical forces induced by nanotopographic structures play a more pivotal role in determining immune responses. Elucidating the mechanotransduction mechanisms by which mechanical forces from nanotopographic structures are converted into intracellular biochemical signals in immune cells is crucial. This understanding is essential for the precise regulation of immune responses mediated by nanotopographic structures and for guiding the development of nanotopographic structures with advanced immunomodulatory properties. This review elucidates the impact of nanotopographic structures on cellular mechanical forces and the subsequent activation of mechanosensors. The ensuing mechano-regulatory effects on immune responses are reviewed, and mechanoimmunomodulation is proposed as a strategy for designing nanotopographic structures to modulate immunity. This review contributes to revolutionizing the strategy for

developing nanotopographic structures and promotes the application of nanotopographic structures with the mechanoimmunomodulatory property in tissue engineering.

Keywords: Nanotopographic structures, mechanical forces, mechanotransduction, immune responses, mechanoimmunomodulation, tissue engineering microstructures

INTRODUCTION

Cells inhabit an elaborate environment, primarily composed of an extracellular matrix, which exhibits a topography ranging from the nano- to micro-meters[1]. Natural nanotopographic structures elicit vital roles in the activation and functionality of living cells. Inspired by this, synthetically fabricated nanotopographic structures have been employed to modify biomaterial surfaces, aiming to manipulate the fate and function of tissue regenerative cells. For example, nanofibers with nanotube surfaces were reported to promote the adhesion, osteogenic differentiation, and mineralization of mesenchymal stem cells (MSCs), thereby accelerating bone regeneration[2]. Additionally, nanostructured materials have been demonstrated to promote the adhesion of vascular cells[3], and nanofibers have been applied in vascular grafts[4]. Thus, nanotopographic structures represent a promising strategy for enhancing tissue engineering outcomes.

Among all the regulatory effects on regeneration-related events and cells, particular attention has been directed towards their impact on immune response modulation and cell behavior. From the initial stage of injury to the completion of healing, immune responses are involved throughout the process and play a critical role in determining the outcome of tissue repair and regeneration[5]. Compared to the direct modulation of functional cells in tissue engineering, nanotopographic structures-induced immune responses can exert a more profound influence. It has been reported that nanotubular surfaces improved implant osteointegration not by promoting the osteogenic activity of MSCs but by modulating macrophage-related inflammation[6]. The Masquelet technique is utilized clinically for treating extensive bone defects[7]. Immune cells that are associated with angiogenesis were activated by the foreign body reaction induced by polymethyl methacrylate which acts as the spacer in the Masquelet technique, initiating a cascade of events that promotes the regeneration of bone with a vascular supply[8,9]. Moreover, unfavorable immune responses can even override the promotion effects of biomaterials on functional outcomes. It was found that the osteogenic differentiation of MSCs induced by β-tricalcium phosphate was negated by significant *in vivo* inflammatory responses, ultimately leading to fibrous encapsulation rather than new bone formation[10]. The immune responses mediated fibrotic cascade where macrophages are necessarily incited by implantable biomedical devices used in clinical settings such as tissue repair, electronic pacemaking, and artificial lens replacement, leads to a risk of device dysfunction and treatment failure[11-14], thereby affecting the therapeutic outcomes. Therefore, it is of great importance to endow nanotopographic structures with favorable immunomodulatory properties in tissue engineering.

To achieve this aim, considerable efforts have been dedicated to the precise fabrication of various complex nanotopographic structures, and an ideal nanotopographic structure is expected to be identified through an empirical "trial and error" approach[15]. With the advancement of nanotechnology, the parameters of nanotopographic structures, including shape, height, width, spacing, and arrangement, are highly customizable, which endows nanotopographic structures with the ability to modulate immune responses[16]. Nonetheless, significant knowledge gaps persist regarding the immune responses to nanotopographic structures[17]. Moreover, the immunomodulatory effects of nanotopographic structures reported are ambiguous and unpredictable. Different surface patterns, including those with similar structures, can demonstrate conflicting effects on immune responses[16]. Therefore, precise immune regulation based on

nanotopographic structures has not yet been achieved. These results imply that the general or fundamental biological mechanisms underlying nanotopographic structures-mediated immune regulation may require further investigation and optimization.

Some studies have revealed that nanotopographic cues can be translated into absorbed protein biosignals or other physicochemical signals, including roughness and wettability[18-21], which are then recognized by immune cells, thus regulating immune responses. For instance, nano-phase alumina increases the absorption of calcium, leading to alternations in the conformation of adsorbed vitronectin and the exposure of additional cell-adhesive epitopes, ultimately facilitating osteoblast adhesion[22]. A common principle observed across these studies is that the initial translation of nanotopographic signals into other types of signals occurs before they are recognized by immune cells, a phenomenon we define as "indirect effects".

In addition to these indirect effects, it has been proved that nanotopographic structures can "directly" deform the cell membrane. Upon membrane deformation, mechanical forces are generated at the membrane due to its viscoelasticity. Some high-aspect-ratio nanotopographic structures, such as nanowires, can even penetrate the cell membrane due to significant concentrated tension[23]. Our previous study has shown that nanotopographic structures with low aspect ratios (ranging from 0.33 to 0.51), including nanorods and nanohemispheres, can deform the cell membrane and generate sufficient contact pressure to initiate cell responses[24]. Mechanical forces are recognized by mechanosensors or immune receptors and are then transduced into biochemical signals that tune cell responses. Generally, compared to some biochemical signals of engineered nanotopographic structures, which are localized and limited in duration due to enzymatic degradation or circulation, mechanical force signals from nanotopographic structures on non- or slow-degradable materials have a more prolonged effect over time and space, continuously stimulating immune cells[25], thus playing a crucial role in immune regulation. This suggests that mechano-regulation may be a more direct and general mechanism for modulating immune responses by nanotopographic structures. Developing advanced nanotopographic structures based on mechanoimmunomodulation should be a promising strategy for achieving precise immune regulation.

Various nanotopographic structures, such as nanotubes, nanorods, nanopores, and nanogrooves, have been shown to modulate immune responses, with the underlying mechanism remaining not well-understood[16]. This results in the inability to achieve precise immune regulation based on nanotopographic structures. Mechano-regulation is widely involved in immune responses, which can affect the movement, activation of immune cells, downstream signal transduction, cell-fate decisions, and effector functions[26-28], thus playing a crucial role in immune modulation. This review focuses on dissecting nanotopographic structures-mediated immune modulation from the perspective of mechanical forces generation, transduction, and the subsequent effects on immune regulation. Therefore, this review first elucidates the generation of mechanical forces on the membrane upon different nanotopographic structures and analyzes the contributory factors. It then discusses the activation modes of mechanosensors through which mechanical forces are transduced into intracellular biochemical signals and further reviews their downstream effects. Subsequently, the mechanism underlying the mechano-regulation of immune responses by nanotopographic structures is summarized. Finally, mechanoimmunomodulation is proposed to recognize the importance of the mechanical forces on nanotopographic structures-mediated immune response. Corresponding strategies for developing advanced nanotopographic structures that induce desired immune responses based on mechanoimmunomodulation are further discussed. The authors' ambition is that this review will contribute to revolutionizing the development strategy for nanotopographic structures and promote the application of nanotopographic structures with mechanoimmunomodulatory properties in tissue engineering.

NANOTOPOGRAPHIC STRUCTURES MEDIATED MECHANICAL CUES ON THE IMMUNE CELL MEMBRANE

To reveal the mechano-regulation mechanism underlying nanotopographic structures mediated immune responses, it is essential first to figure out how mechanical forces are generated at the immune cell membrane after interaction with nanotopographic structures. Cytoskeletal dynamics endow immune cells with viscoelasticity[29], allowing mechanical forces to arise from membrane deformation induced by nanotopographic structures. Under different conditions, the dominant mechanical force varies with changes in the parameters of nanotopographic structures. Here, the cellular structural basis for generating mechanical forces is first introduced, followed by further elucidation of the generation mechanisms of traction force, membrane tension, and contact pressure, respectively. Finally, the effects of the various structural parameters on mechanical forces are discussed.

Cellular structure basis for the generation of mechanical forces mediated by nanotopographic structures

According to our previous study, during the cell landing and statical contact process, the interaction between cells and topographic structures occurs without the participation of cell-autonomous movement, and there was hardly any membrane deformation[24]. The generation of sufficient and effective mechanical forces from nanotopographic structures requires the contribution of intracellular forces that act reciprocally or additively, usually after cell adhesion, as the cell deforms to adapt to the nanotopographic structures.

Intracellular cytoskeletal dynamics serve as the basis for generating intracellular forces. Upon contact with biomaterial surfaces, the interaction between extracellular matrix proteins and adhesion-related proteins such as integrins triggers the activation of cytoskeleton-related proteins, initiating the construction of actin filaments through processes including nucleation, elongation, and capping. Additionally, cortical actin, a loosely organized network formed by actin filaments, contributes to the mechanical strength of cells[30]. Traction force is generated by the actin filaments during myosin contraction, and the outward assembly of actin filaments can produce a protrusive force[31,32] [Figure 1].

Traction forces resulting from nanotopographic structures on immune cell membranes

It has been proposed that nanotopographic structures such as grooves, grids, and pits reduce the area of available adhesive ligands, and act as spatial confiners for integrin binding and the focal adhesion complex, thereby reducing cytoskeletal assembly[33]. This is further supported by the discovery that rigid nanopillars resulted in a smaller focal adhesion complex compared to a flat surface[34]. However, this hypothesis fails to explain why many nanotopographic structures can enhance focal adhesion formation and cytoskeletal assembly. For instance, on nano gratings with a 250 nm line width, focal adhesion kinase exhibited greater elongation compared to a flat surface, and cellular stress fibers showed a more aligned arrangement[35]. Nanopores with a pore diameter size of 20 ± 5 nm also promote cell adhesion[36,37].

Researchers conducted a sophisticated experiment where they fabricated rigid surfaces with nanodots functionalized with integrin ligands. By increasing the spacing between nanodots from 30 to 100 nm, the size of the focal adhesion complex was found to be significantly smaller than that on the rigid surface. Computational modeling results indicated that wider spacing on rigid surfaces resulted in excessive traction force on individual integrins, leading to adhesion collapse, which is akin to the slip bond mechanism. Conversely, wider spacing on low-rigidity substrates exhibited stronger focal adhesion compared to denser patterns[38]. This could be attributed to the relaxation of traction force on low-rigidity substrates, preventing adhesion collapse and enabling the recruitment of more adhesion clusters to reduce the traction force loading on each adhesion molecular clutch[39]. Moreover, it has been proved that the traction force of cells on nanotopographic structures can alter, which is greater than that on flat structures [Figure 2][40].

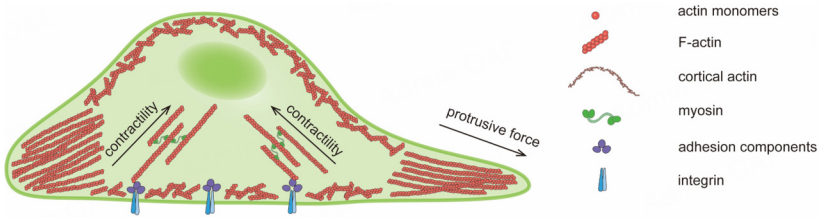

Figure 1. The schematic diagram of the cellular structural basis for generating mechanical forces. The cortical actin, formed by the loose organization of actin filaments, endows immune cells with mechanical strength. Integrins are important molecules in cell adhesion, linking the intracellular cytoskeleton to the extracellular matrix. Large assemblies of actin filaments in stress fibers, along with the myosin, enable the generation of contractility. Organizing actin filaments outward results in protrusive force.

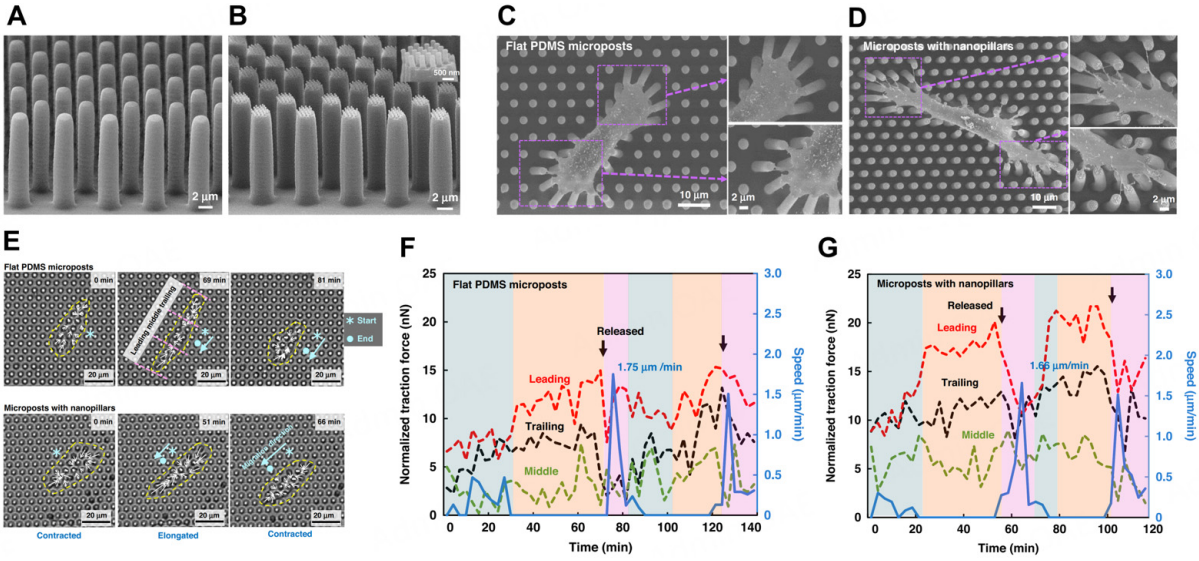

Figure 2. Traction forces resulting from nanotopographic structures on cell membranes. (A) Flat micropost array, which is flexible under cellular traction and can be used to detect the cellular traction force. (B) Microposts with nanopillar. (C and D) Cell exerts traction force that induces deflection of the micropost array and the microposts with nanopillar. (E-G) Cells exert greater traction force on the microposts with nanopillars than the Flat microposts. This figure is quoted with permission from Cheng *et al.*[40].

Therefore, changing traction forces on the membrane is the essential mechanism by which topographic patterns modulate cellular biological behaviors [Figure 3A].

Tensions resulting from nanotopographic structures on immune cell membranes

Deformation of both the cell membrane and nucleus was notably observed on surfaces featuring nanopillars and nanowires[41,42] [Figure 4]. A similar level of cell membrane deformation was observed on a nanowire array with a diameter of approximately 50 nm and a height of around 20 μm[43]. The cell membrane exhibits solid elastic mechanical behavior[44]. Therefore, during the deformation process, mechanical force generation at the membrane occurs. It was observed that when cells adhered to vertically aligned nanowires, their cell membrane undergoes substantial deformation around them, resulting in localized stress concentration, which can even lead to cell membrane penetration in some cases[23].

The protrusive force generated by actin assembly may significantly contribute to driving cell membrane deformation into the spaces between the nanostructures, leading to the aggregation of membrane tensions[39]. In certain widely spaced nanotopographic structures, the cell membrane undergoes substantial deformation and may adhere to the substrate between these structures, thereby reinforcing and sustaining

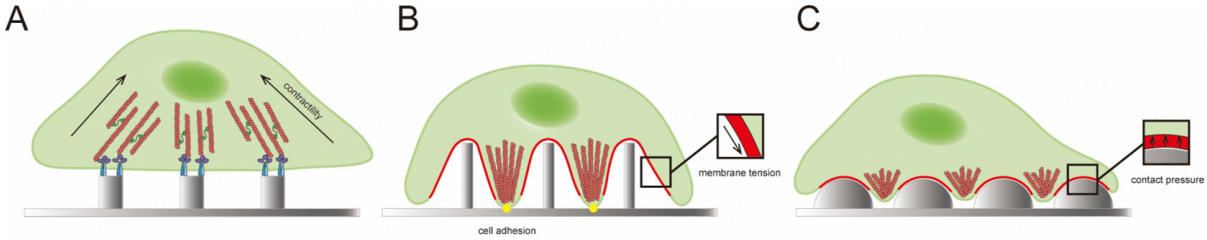

Figure 3. The schematic diagrams of mechanical forces resulting from nanotopographic structures on the immune cell membrane. (A) Traction forces resulting from nanotopographic structures on immune cell membranes due to the contractility of the actin filament; (B) Tensions resulting from nanotopographic structures on immune cell membranes. Actin filaments drive cell membrane deformation into the interspace between nanostructures and adhere to the substrate. Nanotopographic structures act as spatial barriers for cell membranes, thereby inducing the generation of membrane tension that horizontally stretches cell membranes; (C) Contact pressures resulting from nanotopographic structures on immune cell membranes. Actin filaments drive similar membrane deformation into the interspace between nanostructures, pushing the cell membrane to compress the nanostructures. In response, the nanostructures produce a reciprocal force perpendicular to the cell membrane, known as contact pressure.

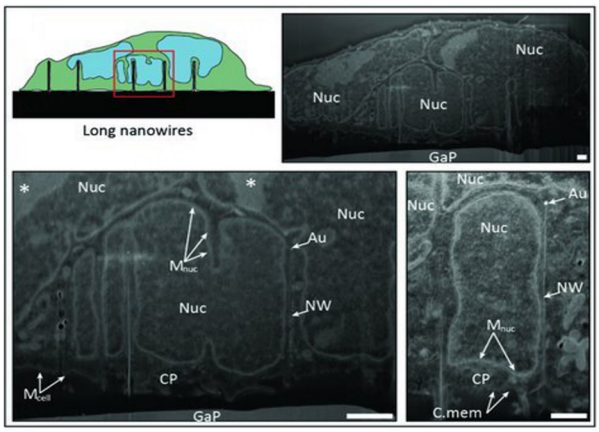

Figure 4. Tensions resulting from long nanowires induce deformation of the cell membrane and nuclear membrane. The nuclei, nuclear membrane, and outer cell membrane are labeled as Nuc, M nuc, and M cell, respectively. The cytoplasm is labeled CP, and areas marked with an asterisk refer to DNA-dense. The gold nanoparticles applied to seed nanowire growth were clearly seen (Au), and the nanowires were also visible (NW). The GaP substrate is labeled GaP. Scale bars 1 μm. This figure is quoted with permission from Persson *et al.*[42].

the membrane tension induced by the nanotopographic structures. In these cases, nanotopographic structures act as spatial barriers to cell membrane deformation, ultimately resulting in the generation of membrane tensions [Figure 3B].

Contact pressures resulting from nanotopographic structures on immune cell membranes

During cell migration, actin polymerization propels the formation of filopodia and generates contractile pressure on the cortical cytoskeleton between filopodia[32]. Consequently, the cellular protrusion driven by actin polymerization is associated with compression of the intermediate area between the protrusions. On nanotopographic surfaces, we observed that the cell membrane protrudes into the spaces among nanostructures[24]. In addition to membrane tension, three-dimensional (3D) finite element analysis results confirmed that nanotopographic structures induce cell membrane deformation, which also exerts contact pressure on the membrane[24] [Figure 5].

Unlike membrane tensions, contact pressures at the membrance often arise from compression between the cell membrane and the vertically oriented nanotopographic structures [Figure 3C]. Membrane tensions and

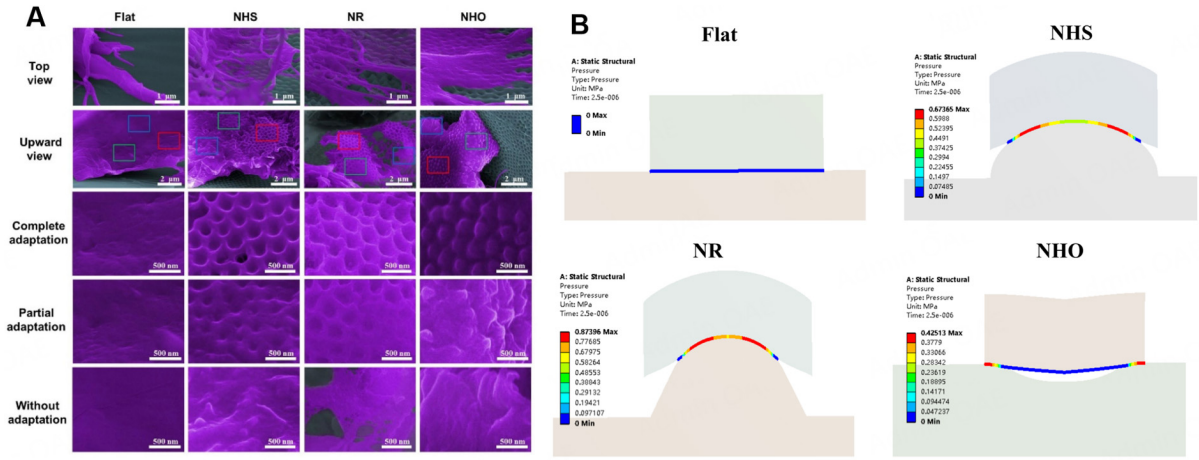

Figure 5. Contact pressures resulting from nanotopographic structures on cell membranes. (A) The SEM image shows mild membrane deformation on some nanostructures; (B) Three-dimensional finite element analysis results confirm that nanotopographic structures exert contact pressure at the membrane. This figure is quoted with permission from Guo *et al.*[24].

contact pressures often coexist, with their relative importance depending on the specific circumstances, including the area of contact involved. A limited contact area may result in a more pronounced increase in the membrane tension, while a sufficient contact area may lead to a more noticeable rise in the contact pressure.

Parameters of nanotopographic structures modify the generated mechanical forces on the immune cell membrane

In recent decades, considerable advancements have been made in the field of nanotopographic structure fabrication techniques, encompassing both top-down and bottom-up methods, as elucidated in our prior review[16]. Currently, nanotopographic structural parameters, including shape, height, width, spacing, and arrangement, exhibit a high degree of customization. This affords nanotopographic structures significant potential for flexible modulation of mechanical cues through precise tuning of these structural parameters. Although achieving precise control over the intricate mechanical cues on immune cell membranes remains challenging, we have nonetheless observed noteworthy correlations between variations in structural parameters and their resultant-induced mechanical cues.

The sharp, angular morphology of nanostructures facilitates the accumulation of tensions within the cell membrane, whereas rounded and smooth structures have a propensity to induce membrane contact pressures. Elevated structures reduce the availability of adhesive sites within the interspace, thereby creating a spatial confinement effect and increasing the traction force exerted per integrin at the membrane interface. As the cell membrane deforms within the interspace, there is a tendency for membrane tension to increase. Regarding width, broader features provide more adhesive sites per unit area, leading to a lower traction force per integrin at the membrane compared to narrower features.

Greater spacing between features enhances the availability of the interspace for adhesion, thereby mitigating the spatial confinement effect on adhesion. Membrane deformation and resistance from the features contribute to increased membrane tension, thereby enhancing traction force loading on integrins within the interspace. Moderate spacing introduces a spatial confinement effect on adhesion, leading to heightened traction force loading per integrin. Conversely, on surfaces with narrow spacing, the spatial confinement effect on adhesion is diminished, potentially promoting increased membrane contact pressure. In terms of

arrangement, disordered patterns augment the distribution of integrins experiencing traction force across specific regions, thereby reducing the traction force loading per integrin within these regions.

However, the shape, height, width, spacing, and arrangement of topographic structures collectively and comprehensively influence the mechanical forces exerted on immune cell membranes, rather than influencing them independently. For instance, while higher features generally augment traction force loading per integrin, widening the structures and reducing the spacing can counteract this effect. Generally, narrow, tall, and moderately spaced features exhibit a strong spatial confinement effect that significantly reduces the available adhesive sites[33], which tends to mainly increase traction force loading per integrin [Figure 6A]. In these cases, decreasing the width and degree of disorder, or increasing the spacing, would further enhance the traction force loading per integrin until the cell membrane deforms into the interfaces between features to obtain more adhesive sites. Conversely, increasing the width, degree of disorder, or decreasing spacing helps to eliminate the spatial confinement effect, thereby reducing the traction force loading per integrin.

Narrow, high, and widely spaced features can induce deformations in the cell membrane at the interfaces between the features, resulting in more adhesive sites and the elimination of the spatial confinement effect. Consequently, these features predominantly induce an accumulation of membrane tension [Figure 6B][23]. Under these circumstances, height is positively correlated with membrane tension, while spacing is negatively correlated. Sharper nanotopographic features can help to increase local membrane tension. Moreover, variations in width and arrangement may not significantly affect membrane tension as much as height, spacing, and shape do.

Moreover, obtuse shapes, low aspect ratios (height/width), and closely spaced features contribute to the generation of membrane contact pressure[24]. In these cases, the aspect ratio of height to width positively correlates with membrane contact pressure. Increasing the spacing would decrease the area under membrane contact, thereby negatively affecting the pressure. Furthermore, changing the shapes of nanotopographic features from obtuse to sharp can reduce the contact area, thereby lowering the membrane contact pressure [Figure 6C].

The precise numerical thresholds of various topographic structural parameters and their impact on mechanical cues have yet to be definitively established, as they vary depending on the cell type and substrate material. The overarching principles outlined here serve as valuable guidelines for the design of topographic parameters; however, specific values may require empirical testing based on the particular cell and substrate materials employed.

TRANSDUCTION OF MECHANICAL FORCE SIGNALS AND THE DOWNSTREAM SIGNALING EFFECTS

Mechanical forces must be recognized by cells before they influence immune responses. Mechanosensors are responsible for transducing mechanical forces and turning them into biochemical signals[45]. Integrins and mechanically sensitive ion channels are the dominant mechanosensors engaged in immune responses[46,47]. Caveolae, an intrinsic subdomain of the cell membrane, can also respond to mechanical forces[48]. Other mechanotransducers involved in immune responses such as the Hippo signaling[49,50] and the T cell receptor (TCR)[26,51] have been reviewed elsewhere.

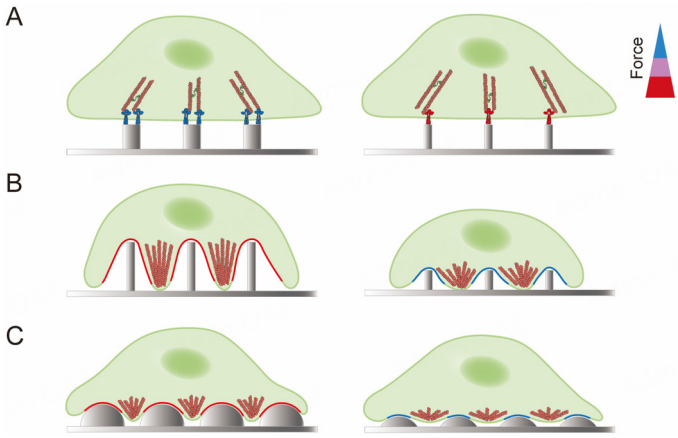

Figure 6. Schematic diagrams for alteration in parameters of nanotopographic structures affecting mechanical forces on the immune cell membrane. (A) Reducing the top surface area of a single rod decreases the number of the adhered integrins, thus increasing the traction force loading on individual integrins, which may lead to adhesion collapse; (B) Lowering the height of nanotopographic structures diminishes the membrane tension; (C) Decreasing the contact area via changing the topographical surface reduces the contact pressure on in the immune cell membrane.

Immune responses form a complex network through the culminating reactions of many immune cells, such as macrophages, T cells, B cells, *etc*. These immune cells exhibit function diversity and plasticity. Mechanosensors mediate the mechanotransduction of mechanical cues, thus regulating the subsequent immune cell responses[52]. Here, the activation modes of the three major mechanosensors and their downstream effects on immune regulation are reviewed.

Activation mode of mechanosensitive ion channels and the downstream effects on immune responses

Mechanosensitive ion channels are a class of ion channels capable of sensing and responding to changes in cell membrane mechanics. Among them, transient receptor potential (TRP) channels and piezoelectric channel proteins (Piezo) are deeply involved in immune responses[53,54]. The TRP vanilloid (TRPV) and Piezo1 channels mediate mechanotransduction by controlling Ca^{2+} influx, and immune cell responses are altered as a result.

Activation mode of TRPV channels and the downstream effects on immune responses

TRP channels are a type of non-selective mechanosensitive cation channels that play a crucial role in sensing mechanical signals in various pathological and physiological processes[55]. Currently, approximately 30 different TRP channels have been successfully identified, and based on sequence homology, they can be divided into six major subfamilies: TRP canonical (TRPC), TRPV, TRP melastatin (TRPM), TRP polycystin (TRPP), TRPM-like (TRPML), and TRP ankyrin (TRPA)[56]. Among them, TRPV channels are the most studied ion channels that mediate the transduction of mechanical signals and ultimately modulate the immune response[57]. TRP channels possess six transmembrane domains with a cation-permeable pore region between the fifth and sixth transmembrane domains[58] [Figure 7]. The opening of this pore can be regulated by mechanical signals, such as membrane tension and cytoskeletal tension[60]. Cellular indentation and membrane stretch are commonly reported to activate TRPV4[61,62].

TRPV4 expression appears to be limited to a few myeloid immune cells[63]. It has been reported that TRPV4 is closely correlated with macrophage function. Dutta *et al.* found that TRPV4 mediated the matrix stiffness-induced M1 polarization both *in vitro* and *in vivo*[64]. Another study also confirmed that TRPV4 controlled the M1 polarization under mechanical stretch stimulation[65]. TRPV4 mediates Ca^{2+} influx[66]

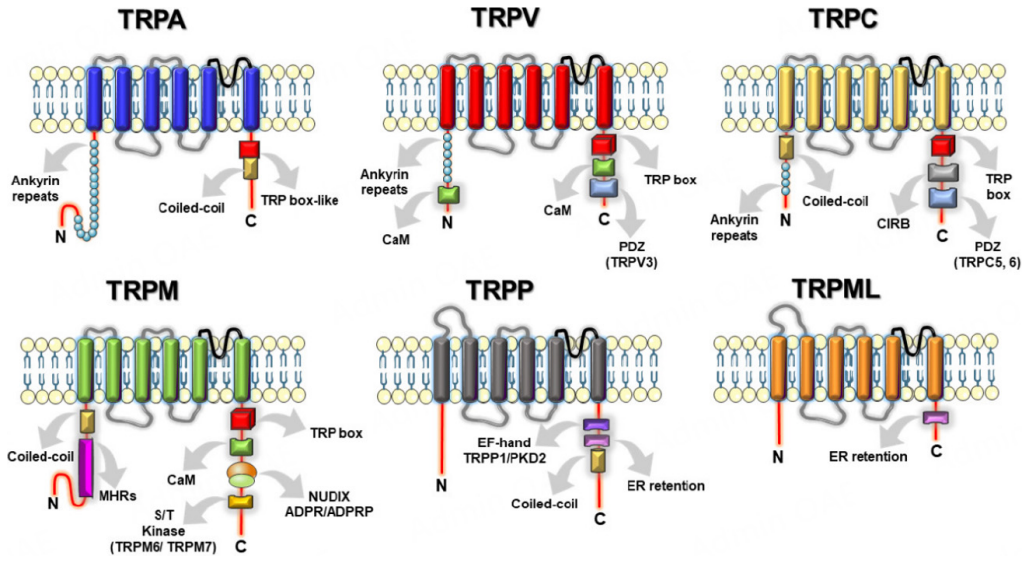

Figure 7. Schematic diagram of TRP channels. TRP channels consist of six transmembrane domains, with a cation-permeable pore loop between the fifth and sixth domains. This figure is quoted with permission from Méndez-Reséndiz *et al.*[59].

[Figure 8], which plays a crucial role in TRPV4-mediated signal transduction. It was reported that TRPV4-mediated Ca^{2+} influx promoted oxidized low-density lipoprotein (LDL) uptake, leading to macrophage foam cell formation[66], and increased the expression of nuclear factor of activated T-cells (NFAT) and nuclear factor kappa B (NF-κB), thus contributing to sustained macrophage activation and inflammation[67,68]. Moreover, given the close correlation between TRPV and inflammation, TRPV inhibition has been proposed as a treatment for many inflammatory diseases, including osteoarthritis[69,70], atherosclerosis[71], and cancer[72].

Activation mode of the Piezo1 channel and the downstream effects on immune responses

Piezo channels are a newly discovered class of mechanosensitive membrane proteins that mainly include two structurally and functionally similar subtypes, Piezo1 and Piezo2[73]. These channels exhibit a certain degree of ion selectivity, with permeable ions primarily Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+}[74]. Cryo-electron microscopy results showed that Piezo1 has a trimeric propeller-like structure, with peripheral regions including "blades", "long beams", and anchoring sites, while the center features a "channel" structure composed of the C-terminal extracellular domain (CED), helical transmembrane domains, and intracellular C-terminal domain (CTD)[75]. Under the resting state, Piezo channels cause the phospholipid membrane to bend locally toward the cell interior. When tension is applied to the cell membrane surface, the central channel structure domain of Piezo channels flattens and expands, promoting channel opening and cations influx [Figure 9][76].

Piezo1 is transcriptionally expressed in numerous immune cells. Piezo1 ion channels are involved in macrophage activation due to their co-localization and coordination with Toll-like receptor 4 (TLR4)[77]. It was reported that Piezo1 promoted stiffness-mediated M1 polarization after stimulation with interferon-gamma (IFN-γ)/lipopolysaccharide (LPS) by increasing Ca^{2+} influx[78]. Another study found that Piezo1-mediated Ca^{2+} promoted NF-κB activation[79]. In addition, Solis *et al.* found that cyclic hydrostatic pressure can promote the opening of Piezo1 channels on the surface of macrophages, leading to Ca^{2+} influx, which, in turn, promoted the synthesis and secretion of endothelin 1 (Edn1) through the phosphorylation of c-Jun, activating hypoxia-inducible factor-1α (HIF-1α)[80]. This activation further up-regulated the expression of inflammatory factors such as interleukin-1beta (IL-1β), C-X-C motif chemokine ligand 10 (CXCL10), and

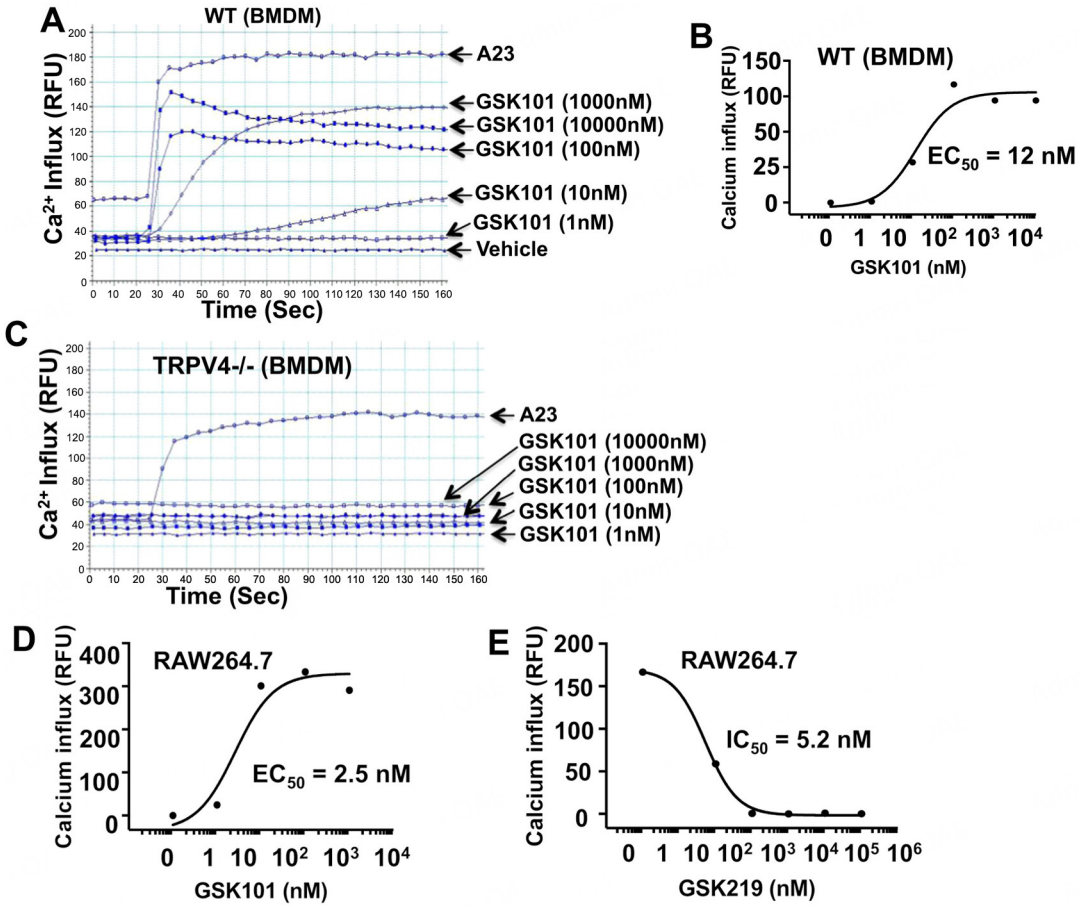

Figure 8. TRPV4 mediates calcium influx in primary normal bone marrow-derived macrophages (BMDMs) and in RAW 264.7 cells. (A-C) Recordings of Calcium 6 dye-loaded BMDMs showing the effect of the TRPV4 agonist GSK101 on Ca^{2+} influx in WT and TRPV4-/- cells; (D and E) Dose-dependent Ca^{2+} influx elicited by GSK101 and GSK219 in RAW 264.7 cells through TRPV4. This figure is quoted with permission from Goswami *et al.*[66].

prostaglandin-endoperoxide synthase 2 (PTGS2)[80]. When Piezo1 was knocked out in mice, the enhancing effect of cyclic hydrostatic pressure on pulmonary inflammation was significantly suppressed, indicating the important role of Piezo1 in sensing cyclic hydrostatic pressure signals and regulating inflammatory responses[80]. In line with that, another study found that high fluid shear forces caused by aortic valve stenosis can be sensed by Piezo1, resulting in increased Ca^{2+} influx and a significant elevation in the expression of inflammatory factors such as IL-1β, interleukin-6 (IL-6), and interferon-beta1 (IFN-β1) in monocytes[81]. These results confirm the crucial role of Piezo1 in inflammation and suggest it as a candidate target in the treatment of inflammation-related diseases. Aykut *et al.* discovered that peptide inhibitors of Piezo1 protein exhibited therapeutic effects in septic shock and can also improve the survival rate of patients with sepsis[82], confirming the feasibility of manipulating Piezo1 to treat inflammation-related diseases.

In addition to macrophages, Piezo1 is also involved in the activation and functionality of B and T cells. Generally, B cells are activated by B cell receptor (BCR) recognizing membrane-presented antigens, and it has been reported that traction force is involved in B cell activation[83]. Moreover, Wan *et al.* found that the mechanical force thresholds required to activate different BCRs vary, which may account for the differences in reaction sensitivity to antigens between naïve and memory B cells[84]. A recent study reported that Pizeo1-mediated Ca^{2+} influx is required for B cell activation in response to membrane-presented antigens[85]. For

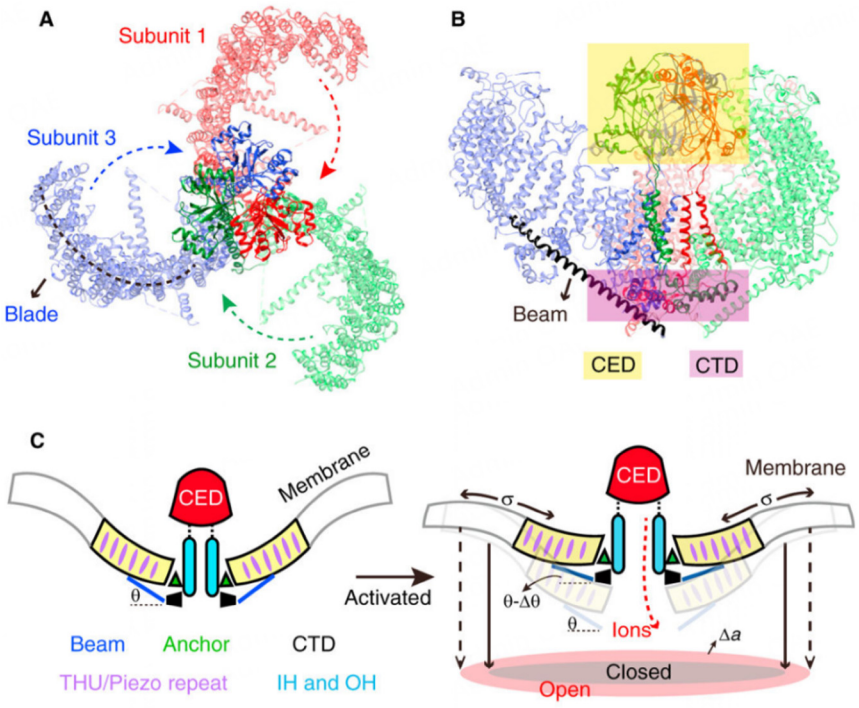

Figure 9. The molecular structure and mechanosensing mechanism of Piezo1. (A) Top view of the molecular structure of Piezo1; (B) Side view of the molecular structure of Piezo1; (C) Piezo dimples the membrane into the cell, with the central channel structure closed (left panel), under low membrane tension. As membrane tension increases, the curved blades of Piezo flatten and the central channel structure opens (right panel). This figure is quoted with permission from Liang *et al.*[76].

T cells, Jairaman *et al.* found that Piezo1 is not required for lymph node homing, TCR-evoked Ca^{2+} signaling in $CD4^+$ T cells, and $CD4^+$ T cell proliferation, but selectively restrains Treg Cells from mice [Figure 10][86]. However, another study reported that Piezo1 promotes optimal mice T cell activation when facing a tumor challenge, while the deletion of piezo1 results in a decreased CD4:CD8 ratio, increased tumor aggression, and higher tumor growth rates[87]. Similarly, piezo1-driven Ca^{2+} influx facilitates TCR signaling and has been found in human T cells[88]. Hope *et al.* also reported that piezo1 is essential for fluid shear stress-induced enhanced human T cell activation and the expression of the cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2), and IFN-γ[89]. These contradictory results may owe to differences in cell sources and external stimuli, which also suggest that there is still much to learn about piezo1 in T cells.

Activation mode of integrins and the downstream effects on immune responses

Integrins are heterodimeric receptors composed of α and β subunits, mediating cell adhesion by linking the extracellular matrix to the cellular cytoskeleton. To date, 18 α subunits and 8 β subunits have been discovered in mammals, forming 24 different integrin heterodimers in total[90]. Currently, the protein structures of some integrin molecules have been successfully identified. Cryo-electron microscopy and X-ray diffraction results have shown that integrin α and β subunits both consist of three main parts: a large extracellular domain, a single transmembrane structure, and a shorter intracellular domain[91]. The extracellular domain contains ligand-binding sites capable of recognizing molecules such as fibronectin and fibrinogen, while the intracellular domain interacts with cytoskeletal proteins such as talin1[92].

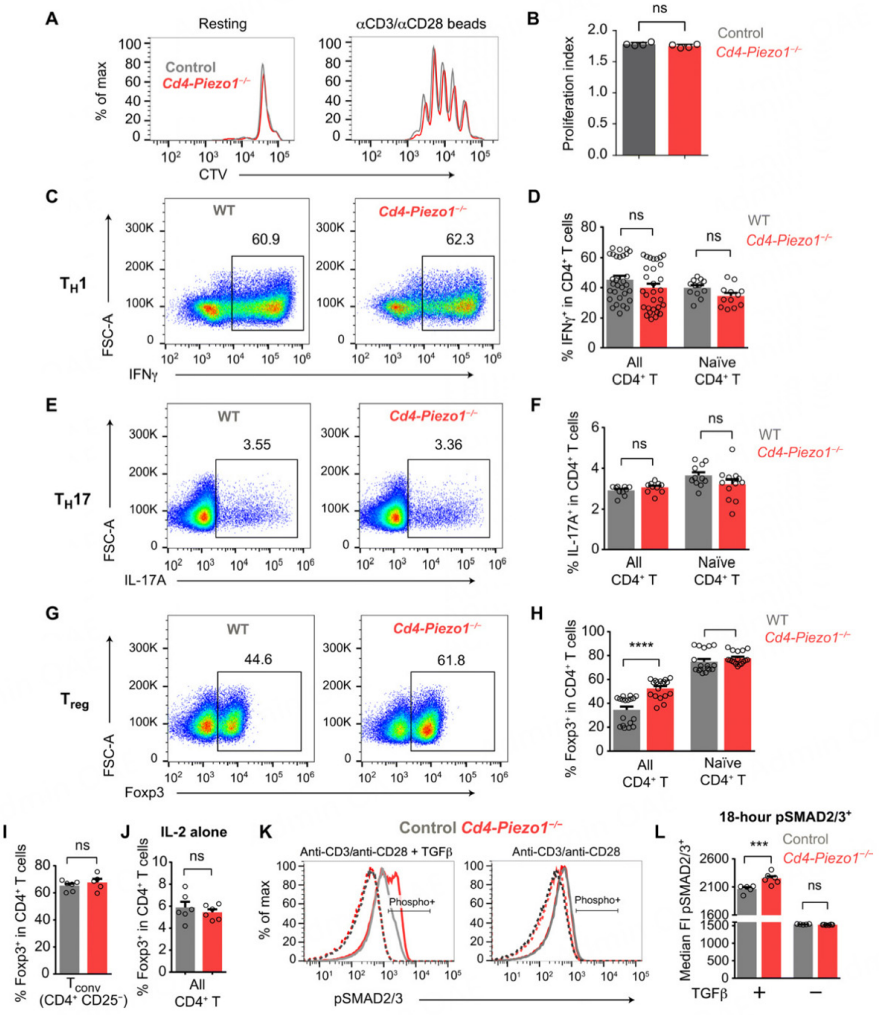

Figure 10. Effect of Piezo1 deletion on proliferation and polarization of $CD4^+$ T cells *in vitro*. (A and B) Piezo1 was not required for $CD4^+$ T cell proliferation; (C-F) Piezo1 does not affect T cell (T_H1 and T_H17) polarization; (G-L) Piezo1 modulates TGFβ signaling and restrains T_{reg} cells (***$P < 0.001$, ****$P < 0.0001$, ordinary one-way ANOVA with Sidak's multiple comparisons, data are means ± SEM, $n > 5$ samples). This figure is quoted with permission from Jairaman *et al.*[86].

Under the resting state, the extracellular domain of integrins exhibits a bent conformation, resulting in low ligand-binding affinity and signaling activity. When activated, the extracellular domain undergoes conformational extension, leading to a significant increase in ligand-binding affinity and signaling activity[93] [Figure 11]. In addition to ligand signals, the activation and function of integrins are also regulated by mechanical forces[94]. It has been reviewed that stretch forces, fluid shear forces, and intracellular osmotic pressure can all be sensed by integrins, leading to changes in integrin activation degree[95]. Stiffness also tunes the activation of integrins. Contractility of the intracellular cytoskeleton can transmit outward through integrins, and extracellular matrix with high stiffness can quickly resist the contraction, contributing an enhanced reciprocal traction force, which can be sensed by integrins and induce conformational changes and activity alterations of integrins[96].

In terms of the reaction to mechanical forces, two types of bonds that mediate the connection of integrins-ligands or integrins-mechanosensitive adaptor proteins are defined. Slip bond exhibits the behavior where force shortens bond lifetimes that indicate the bonding affinity. In contrast, mechanical forces prolong the

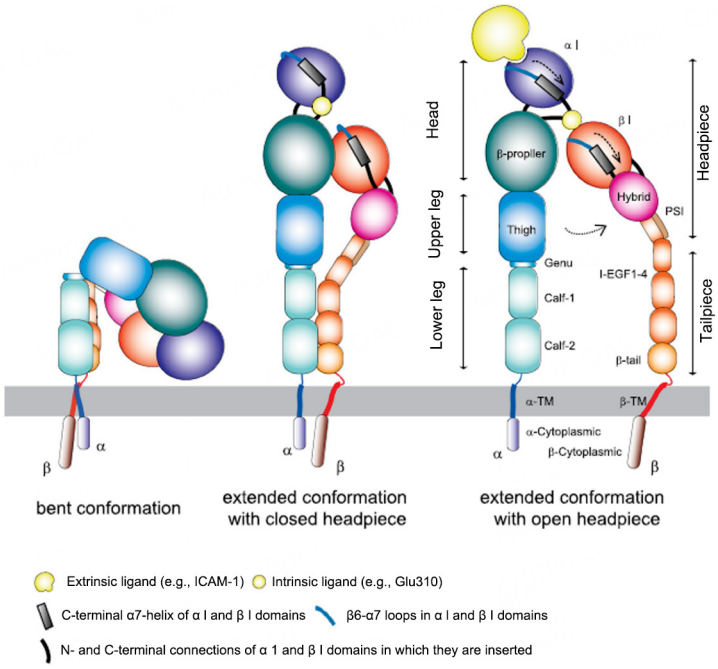

Figure 11. Schematic diagram of the structure of integrins. A bent conformation makes integrins inactive, while an extended conformation makes integrins active and endows integrins with the high affinity for ligands. This figure is quoted with permission from Park *et al.*[93].

bond lifetimes of the catch bond[97,98]. When loaded with forces, slip bond tends to rupture while catch bonds can be allosterically stabilized. Catch bond-dependent adhesion strengthening is necessary to introduce downstream signaling such as activation of focal adhesion kinase (FAK) and nuclear translocation of the transcription factor Yes-associated protein (YAP)[39].

After stable cell adhesion, focal adhesion in immune cells can be observed. Integrins are the dominant components of focal adhesion, linking the intracellular cytoskeleton to extracellular nanotopographic structures[18]. This gives integrins the ability to mediate mechanostransduction of mechanical cues from nanotopographic structures, thereby regulating immune cell responses. Upon activation, integrins recruit various proteins to their short cytoplasmic tails, such as talin and vinculin[99], thereby regulating various downstream signaling transduction pathways, including cytoskeleton activation. Changes in integrin-mediated cytoskeleton function affect the nuclear translocation of mechanotransduction molecules YAP/TAZ (transcriptional coactivator with PDZ-binding motif) and myocardin-related transcription factor A (MRTF-A)[100,101]. Adhesion complexes, cytoskeleton, YAP/TAZ, and MRTF-A are all closely linked to inflammatory responses. For instance, FAK can interact with multiple NF-κB pathway signaling molecules, including inhibitors of kappa B kinase-α (IKK-α), thereby influencing inflammatory responses[102]. Guo *et al.* found that nanotopographic surfaces in low-aspect ratios, such as nanorods and nano hemispheres, elevated contact pressure at the macrophage-nanotopography interface post-adhesion[24]. This impeded the conformational extension and the subsequent activation of integrin β2, thereby down-regulating focal adhesion and the PI3K-Akt signaling pathway. Consequently, NF-κB signaling and the ultimate macrophage inflammatory responses were alleviated[24] [Figure 12]. Many signaling molecules involved in cytoskeleton activity, such as Rho-associated coiled-coil kinase (ROCK), Ras homolog gene family member A (RhoA), and MRTF-A, also contribute to the regulation of inflammatory responses[103]. It was found that titania nanotubes (TNTs) inhibited the nuclear envelope protein lamin A/C, thereby reducing actin

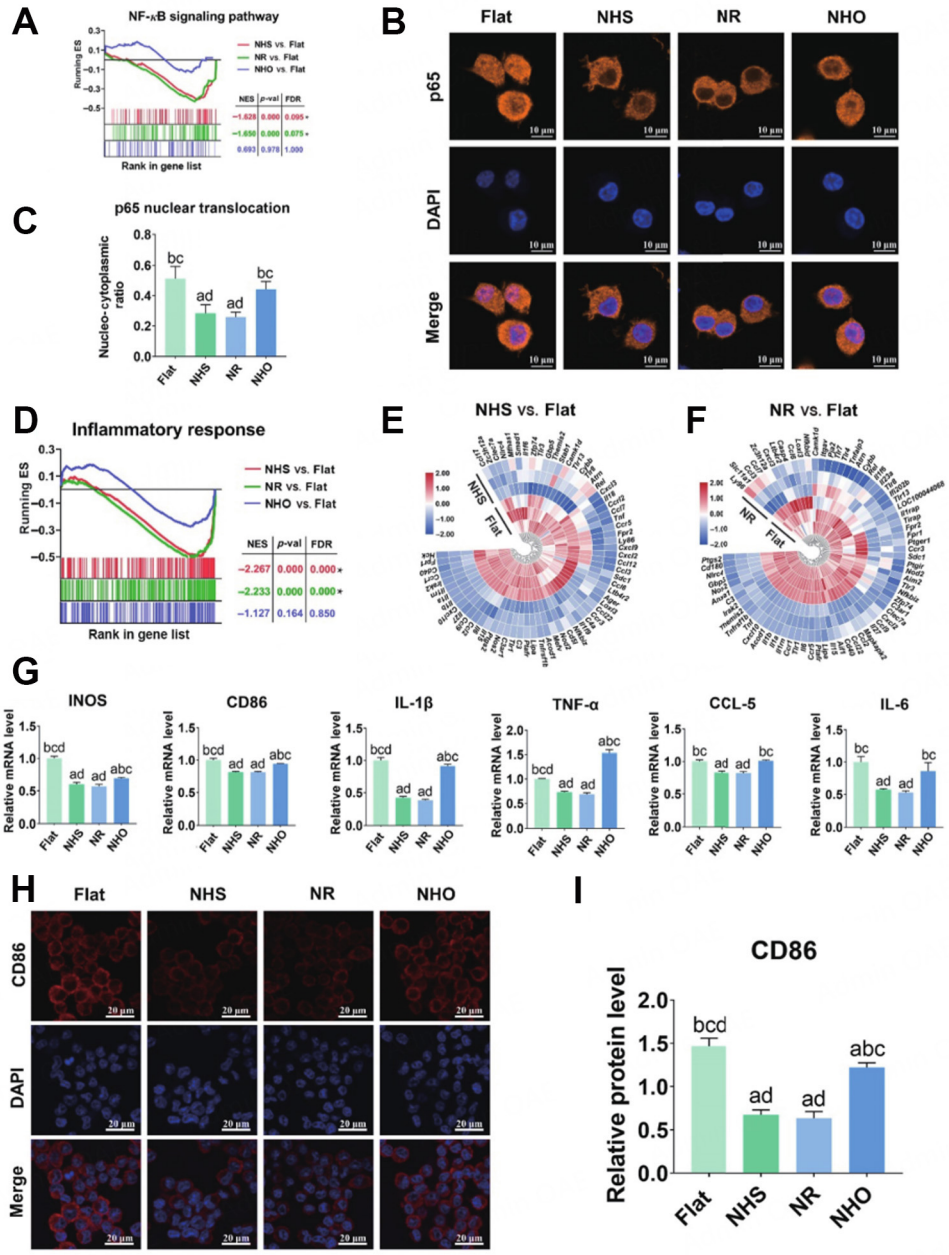

Figure 12. NF-κB signaling and the ultimate macrophage inflammatory responses are alleviated in the nanorod and nanohemisphere groups. (A-C) GSEA results of the NF-κB signaling pathway, representative immunofluorescent staining images of p65 nuclear translocation and it's semi-quantitative statistical analysis result; (D-F) GSEA results of the inflammatory response and the expression heatmap of leading genes across different surfaces; (G-I) RT-qPCR results of several typical inflammatory genes, representative immunofluorescent staining images and semi-quantitative statistical analysis of the expression of CD86 across different surfaces. Significant differences between two groups are defined by $P < 0.05$ and presented as follows: [a] *vs.* Flat, [b] *vs.* NHS, [c] *vs.* NR, and [d] *vs.* NHO. All data are means ± SD; $n \geq 3$. This figure is quoted with permission from Guo *et al.*[24].

polymerization, which, in turn, limited the nuclear translocation of the actin-dependent transcriptional cofactor MRTF-A, ultimately mitigating inflammation[104]. YAP/TAZ can increase the expression of inflammatory cytokines, such as IL-6[105]. After nuclear translocation, MRTF-A can bind to the promoter regions of inflammation-related genes, promoting the expression of various inflammatory cytokines, such as TNF-α and IL-6[103].

Integrins also participate in B cell responses. The concentration of antigen and the affinity of the BCR define a sharp threshold, which tightly regulates the B cell responses. It was found that the binding of lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) cooperated with the BCR by facilitating B-cell adhesion and conjugate formation, thereby decreasing the thresholds for the B-cell response[106]. Another study reported that activation of LFA-1 induced the formation of a contractile actomyosin arc network, which drove mechanical force-dependent immune synapse (IS) formation, promoting B-cell activation[107]. Moreover, it was reported that germinal center B cell fitness was influenced by integrin $\alpha_L\beta_2$ and $\alpha_4\beta_1$-mediated interactions between B Cells and follicular dendritic cells[108]. Marginal zone B cell differentiation was also found to be regulated by integrin β1[109]. Integrins do not just act as adhesion points for T cells, but also influence T cell functionality[110]. Immunological synapse (IS) is the fundamental structure for T cell activation, featured by the concentration of TCRs at its center surrounded by a ring of LFA-1 ($\alpha_L\beta_2$)[111], making LFA-1 a candidate molecule that can modulate T cell activation. It was reported that activation of LFA-1 via its ligand ICAM-1 slowed the centripetal flow of actin, resulting in a reduction in tyrosine phosphorylation downstream of the TCR[112]. On the other hand, immediate β2 integrin activation upon TCR activation was also observed[113]. Consequently, LFA-1 and TCR exhibit complementary effects in inducing actin dynamics and cytoskeletal tension generation at the IS, which is necessary for full signaling[114]. Recently, it was found that LFA-1 was subjected to an F-actin-dependent pulling force at the IS, and this force enhanced the function of cytotoxic lymphocytes by promoting synaptic degranulation and killing[115].

Activation mode of caveolae and the downstream effects on immune responses

Caveolae are flask-shaped, intrinsic submicroscopic immune cell membrane subdomains with diameters of 50-100 nm, characterized by the presence of the scaffolding protein caveolin[116,117]. Caveolins (Cav) are key functional components of caveolae. There are three caveolin proteins (Cav-1, Cav-2, Cav-3) present in mammals, all of which are integral membrane proteins embedded in the cell membrane through hairpin domains, with their amino and carboxyl termini located within the membrane[118]. Caveolae are involved in the regulation of membrane dynamics and can respond to membrane stress by flattening into the plasma membrane. The disassembly/reassembly cycle of caveolae mediates the mechanotransduction of mechanical cues on the immune cell membrane[119].

Many signal transduction molecules can co-localize with caveolin proteins within caveolae, where caveolins serve as scaffold proteins providing binding sites for signal transduction molecules. Caveolae undergo internalization mediated by proteins such as protein kinase C-α (PKCα) and Filamin A (FLNA), leading to the endocytosis and degradation of various membrane proteins[118]. Upon mechanical stress stimulation, the invaginated conformation of caveolae flattens, and the interactions between caveolin proteins and other caveolar structural components are disrupted, leading to the dissociation and release of multiple caveolar structural components into the cytoplasm[120] [Figure 13]. Caveolae-dependent mechanotransduction plays an important role in the responses of immune cells to external stimuli as well.

Studies have shown that caveolae and caveolin proteins are closely connected with inflammation. It has been reported that many inflammation-related signaling molecules, such as TLR4, endothelial nitric oxide synthase (eNOS), and cyclooxygenase-2 (COX-2)[121-123], are co-localized with Cav-1 and that their activity is inhibited by this interaction. Upon sensing mechanical signals, such as shear stress, pressure, or stretching, these signaling molecules are released, activating the downstream pathways, including the NF-κB signaling pathway[124,125]. Consistently, it was found that the downregulated expression of Cav-1 in mouse alveolar and peritoneal macrophages led to further increases in LPS-induced TNF-α and IL-6 expression, while the production of the anti-inflammatory cytokine IL-10 decreased[126]. Overexpression of Cav-1 in RAW264.7 cells significantly reduced LPS-induced NF-κB and activator protein-1 (AP-1) activations[126]. Therefore, caveolae play an important role in maintaining the homeostasis of the inflammatory responses.

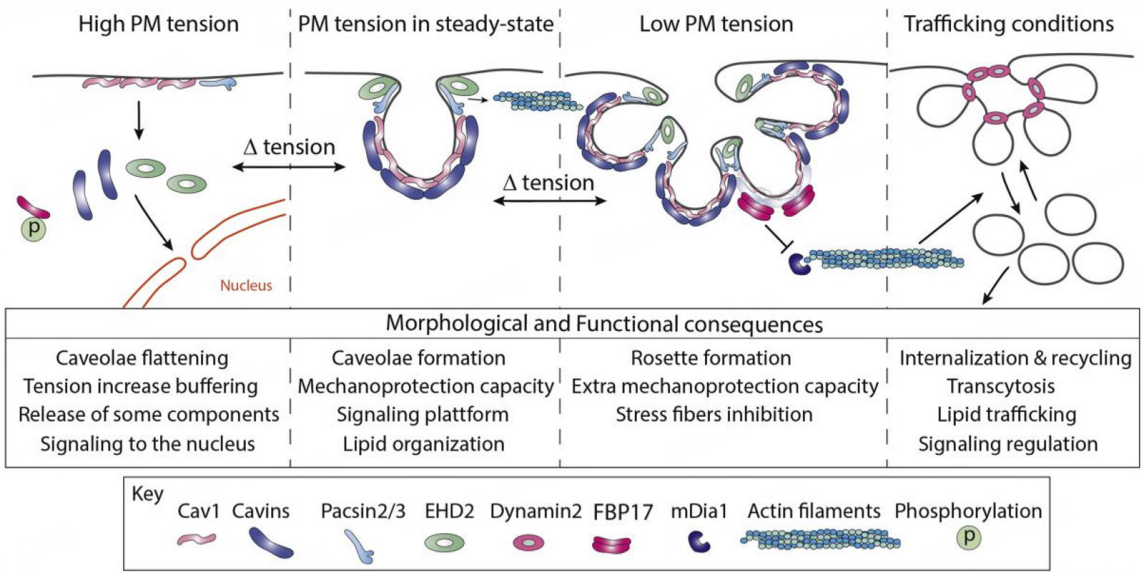

Figure 13. Schematic diagram of disassembly/reassembly cycle of caveolae under different membrane tensions. Many signal transduction molecules can co-localize with caveolin proteins within caveolae. High membrane tension flattens caveolae into the membrane, resulting in the release of signaling components. Low membrane tension contributes to the formation of rosettes, while the loss of adhesion leads to the trafficking of caveolae. This figure is quoted with permission from Del Pozo *et al.*[120].

Caveolae are also found to be involved in B cell activation and functionality. It has been reported that Cav-1 is expressed in human and mouse B cells. Cav-1 deficiency did not affect thymus-dependent immune responses, whereas it reduced IgG (3) secretion *in vitro* in response to LPS[127]. Another study found that Cav1 acts as a cell-intrinsic regulator, preventing B cell-induced autoimmunity by controlling the distribution of isotype-specific BCR nanoclusters[128]. Taken together, caveolae and caveolin proteins also regulate immune responses.

IMPLICATIONS FOR ADVANCING NANOTOPOGRAPHIC STRUCTURES WITH THE "SMART" MECHANOIMMUNOMODULATION PROPERTY

As reviewed above, nanotopographic structures can directly and flexibly modulate mechanical cues on the immune cell membrane including the traction force loading, the membrane tension, and the contact pressure by adjusting parameters such as the shape, height, width, spacing, and their arrangement. Mechanical forces induced by nanotopographic structures change the configuration of mechanosensors, including integrins, Piezo1, TRPV, and Caveolae, at the immune cell membrane. Consequently, this leads to cytoskeleton movement, Ca^{2+} influx, release of signaling components, and activation of TCR or BCR. These changes subsequently activate intracellular signaling pathways, such as the PI3K-Akt, YAP/TAZ, HIF-1α, and NF-κB pathways, ultimately modulating the final immune responses [Figure 14]. These findings are synthesized as the direct mechano-regulation mechanism underlying nanotopographic structures-mediated immune responses, providing new insight into the immunomodulation effects of both natural and engineered nanotopographic structures. It should be noted that the effective mechanosensitive membrane proteins, intracellular mechanotransduction pathways, and immune responses may differ among various immune cells. Only several typical signaling pathways engaged in the mechano-regulation of immune responses have been presented. Other signaling pathways involved in mechanoimmunomodulation require further investigation. In addition to immune responses, other types of cell responses, such as micropinocytosis[129], proliferation, and differentiation[130], can also be influenced by nanotopographic structures. These responses can also influence cell function and should not be overlooked when discussing nanotopographic structures-induced immune cell responses.

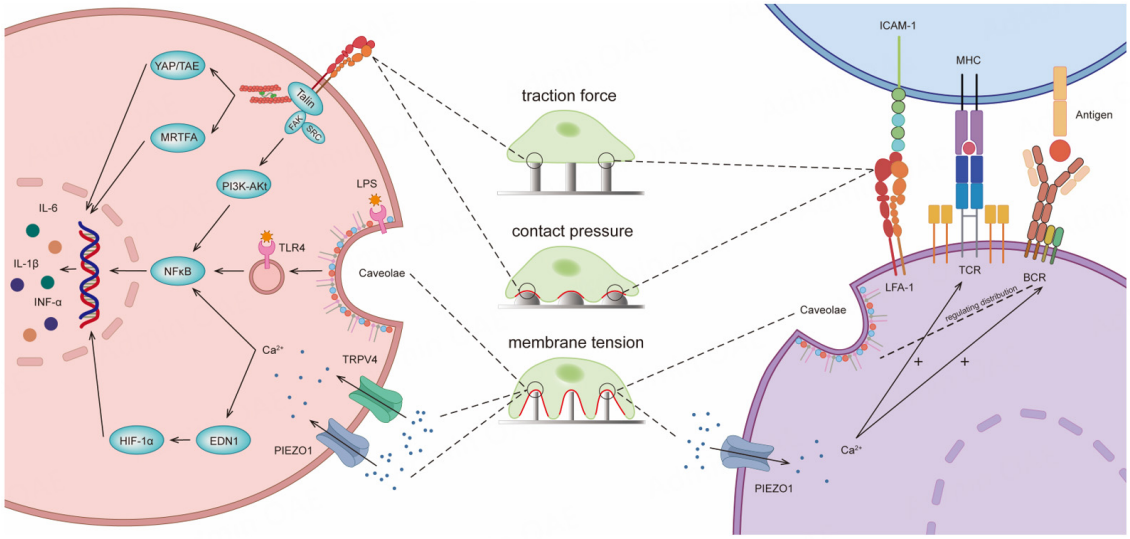

Figure 14. Schematic diagram of the direct mechano-regulation mechanism underlying nanotopographic structure-mediated immune responses. Nanotopographic structures modulate traction force, membrane tension, and contact pressure on the immune cell membrane. Traction force and contact pressure affect the configuration of integrins, recruiting talin to their cytoplasmic tails, thereby regulating cytoskeletal activation. Changes in integrin-mediated cytoskeletal function influence the nuclear translocation of the mechanotransduction molecules YAP/TAZ and MRTF-A, thus regulating the gene expression of inflammatory factors. Alternation of the activity of integrins also affects cell focal adhesion activity, as well as downstream PI3K-Akt signaling and subsequent NF-κB signaling. Additionally, activation of LFA-1 promotes B-cell and T-cell activations. Membrane tension can activate Piezo1, TRPV, and Caveolae. Force-induced opening of TRPV4 and Piezo1 results in Ca^{2+} influx, promoting activation of NF-κB and synthesis of EDN1, which facilitates the accumulation of HIF-1α. NF-κB and HIF-1α translocate to the nucleus, driving the expression of inflammatory genes. Additionally, Piezo1-mediated Ca^{2+} influx can activate TCR and BCR. Membrane tension also leads to the disassembly of caveolae, releasing co-localized, inflammation-related signaling molecules such as TLR4, which activate downstream NF-κB signaling pathways. Additionally, caveolae control the distribution of BCR nanoclusters.

In cognition of the importance of mechanical forces in nanotopographic structures-mediated immune responses, mechanoimmunomodulation has been proposed to guide the development of advanced nanotopographic structures that induce favorable immune responses [Figure 15]. Mechanosensitive membrane proteins serve as the crucial intermediary in the transduction of mechanical cues from nanotopographic structures to biochemical signals in immune cells, playing a pivotal role in mechanoimmunomodulation[45]. Therefore, targeting specific mechanosensitive membrane proteins is a promising strategy for developing nanotopographic structures with favorable immunomodulatory properties. However, the expression profiles of different mechanosensitive membrane proteins vary among different immune cells, and the direction and magnitude of mechanical forces required for activation also differ[131]. To fabricate nanotopographic structures that induce desired immune responses in specific cells, the candidate mechanosensitive membrane proteins should first be identified, followed by decoding the activation mechanisms and downstream effects. Subsequently, based on the requirements, including the magnitude and direction of the mechanical force of the target mechanosensitive membrane protein to elicit desired immune responses, the ideal nanotopographic structures can be rationally designed and then fabricated.

Nanotopographic structures have been extensively applied in fields of clinical medicine, including dentistry, orthopedics, and interventional treatment. For instance, nanotubes, nanowires, and nanohemispheres have been used to modify oral implant surfaces to enhance osseointegration, thus improving clinical performance[132-134]. Nanotopographic structures have also been applied to the surfaces of orthopedic implants to mimic the natural nanostructures of bone tissue, expected to enhance osseointegration with the

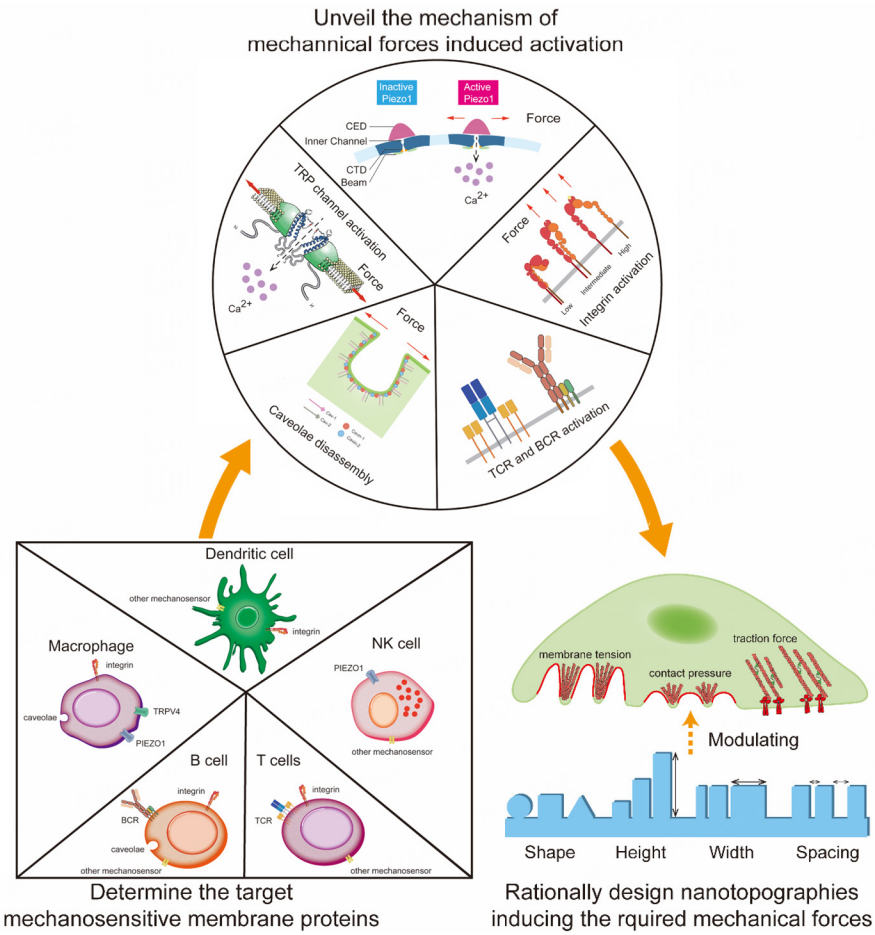

Figure 15. A schematic diagram shows the development of nanotopographic structures based on mechanoimmunomodulation. To fabricate nanotopographic structures that induce the desired immune response in specific cells, the candidate mechanosensitive membrane proteins should be first identified, followed by unveiling their activation mechanisms and downstream effects. Subsequently, based on the requirements, such as the magnitude and direction of the mechanical forces required for the target mechanosensitive membrane proteins to elicit the desired immune response, the ideal nanotopographic structures can be rationally designed by adjusting or combining different parameters, including shape, height, width, and spacing.

host bone tissue[135] and nanofibrous scaffolds have been used for bone tissue engineering[136]. Nanolinear patterns that modify the surface of cardiovascular stents can foster endothelialization without enhancement of platelet adhesion, promising to solve in-stent restenosis[137]. Nanoparticles were also applied for drug delivery when treating cardiovascular diseases[138]. In these clinical applications, immune responses play a crucial role in determining the outcome. Based on mechanoimmunomodulation, the precise immune regulation of engineered nanotopographic structures is expected to be achieved. Subsequently, the performance of engineered nanotopographic structures in tissue engineering will be further improved, and clinical translation will be promoted.

Based on mechanoimmunomodulation, nanotopographic structures that induce favorable immune responses would be achieved. Additionally, with advancements in techniques for mechanical force measurement at the single-cell level or optimizing mathematical calculation[26,139,140], the quantitative assessment of the mechanical forces exerted by nanotopographic structures on the immune cell membrane can be achieved. Subsequently, a comprehensive dataset elucidating the interplay among "nanotopographic structures-mechanical forces-immune responses" can be established. Artificial intelligence can further learn

from this comprehensive dataset and ultimately understand the correlation among nanotopographic structures, mechanical forces, and immune responses via Deep Learning networks. After that, these techniques can be applied to aid in the more efficient screening or design of nanotopographic structures with desired immunomodulatory effects. For example, for requirements of nanotopographic structures with anti-inflammatory effects, artificial intelligence can be applied to identify suitable existing structures or automatically generate new structures by combining various parameters according to the results of Deep Learning. By harnessing artificial intelligence techniques, this dataset holds the potential for promoting the development of advanced nanotopographic structures with the "smart" mechanoimmunomodulation property. Furthermore, advancements in nanotechnology[141] and 3D printing[142] will facilitate the fabrication of intricate topographies capable of multi-regulation, temporal-regulation, and spatial-regulation of immune responses at both two- and three-dimension levels.

CONCLUSIONS

Nanotopographic structures can elicit mechanical forces on the immune cell membrane, which can further alter the configuration of mechanosensitive proteins, thereby activating downstream signaling pathways and regulating immune responses. This process encapsulates the mechanism through which nanotopographic structures mediate the mechano-regulation of immune responses. Accordingly, mechanoimmunomodulation is proposed to guide the development of advanced nanotopographic structures with favorable immunomodulatory properties, emphasizing the importance of mechanical forces in nanotopographic structures-mediated immune responses. Future studies should focus on identifying the dominant mechanosensor of the target cell, followed by the rational design of specific nanotopographic structures based on the required magnitude and direction of the mechanical forces needed to induce the desired immune responses, thus achieving precise regulation of immune responses mediated by nanotopographic structures. By constructing a comprehensive dataset elucidating the interplay among "nanotopographic structures-mechanical forces-immune responses" and combining combining artificial intelligence techniques, high throughput smart development of advanced nanotopographic structures exhibiting appropriate mechanoimmunomodulation can be obtained. These advancements herald a new era in the precise mechano-regulation of immune responses based on nanotopographic structures, and will further promote the application of nanotopographic structures with mechanoimmunomodulatory properties in tissue engineering.

DECLARATIONS

Authors' contributions

Conceptualization and writing-original draft: Ao, Y.; Guo, Y.
Writing-review and editing: Ao, Y.; Xia, R.; Guo, X.
Illustration: Xia, R.; Cai, Y.; Wang, J.
Writing-review & editing, supervision, and funding acquisition: Chen, Z.

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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