

Tools of the trade: podosomes as multipurpose organelles of monocytic cells

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Abstract Podosomes are adhesion and invasion structures that are particularly prominent in cells of the monocytic lineage such as macrophages, dendritic cells, and osteoclasts. They are multifunctional organelles that combine several key abilities required for cell migration and invasion. The podosome repertoire includes well-established functions such as cell-substrate adhesion, and extracellular matrix degradation, recently discovered abilities such as rigidity and topology sensing as well as antigen sampling, and also more speculative functions such as cell protrusion stabilization and transmigration. Collectively, podosomes not only enable dynamic interactions of cells with their surroundings, they also gather information about the pericellular environment, and are actively involved in its reshaping. This review presents an overview of the current knowledge on podosome composition, architecture, and regulation. We focus in particular on the growing list of podosome functions and discuss the specific properties of podosomes in macrophages, dendritic cells, and osteoclasts. Moreover, this article highlights podosome-related intracellular transport processes, the formation of podosomes in 3D environments as well as potentially podosome-associated diseases involving monocytic cells.

Keywords Actin · Dendritic cells · Extracellular matrix · Macrophages · Matrix metalloproteinases · Microtubules · Osteoclasts

Abbreviations

| | |
|---------|---|
| ADAM | A disintegrin and metalloproteinase |
| Arp | Actin-related protein |
| CD | Cluster of differentiation |
| ECM | Extracellular matrix |
| FMNL | Formin-like |
| HDAC | Histone deacetylase |
| KIF | Kinesin-like |
| LAD | Leukocyte adhesion deficiency |
| MMP | Matrix metalloproteinase |
| MT1-MMP | Membrane-type 1-matrix metalloproteinase |
| PAPA | Pyogenic sterile arthritis, pyoderma gangrenosum, and acne (syndrome) |
| PSTPIP | Proline-serine-threonine phosphatase interacting protein |
| PMA | Phorbol 12-myristate 13-acetate |
| SZ | Sealing zone |
| TAM | Tumor-associated macrophage |
| WAS | Wiskott-Aldrich syndrome |
| WASP | Wiskott-Aldrich syndrome protein |

Introduction

Cells form a variety of structures that enable them to adhere to their surroundings, to protrude and migrate on surfaces, and to invade into tissues. These structures include protrusions such as filopodia, lamellipodia, and blebs [1], adhesions such as focal complexes, focal adhesions, and fibrillar adhesions [2], and also invadosomes, the latter comprising podosomes and invadopodia [3, 4].

Within the invadosome group of matrix-lytic adhesions, podosomes embody the more physiological aspect, as they are formed in untransformed cells such as monocytic cells [5–7], endothelial cells [8, 9], smooth muscle cells [10],

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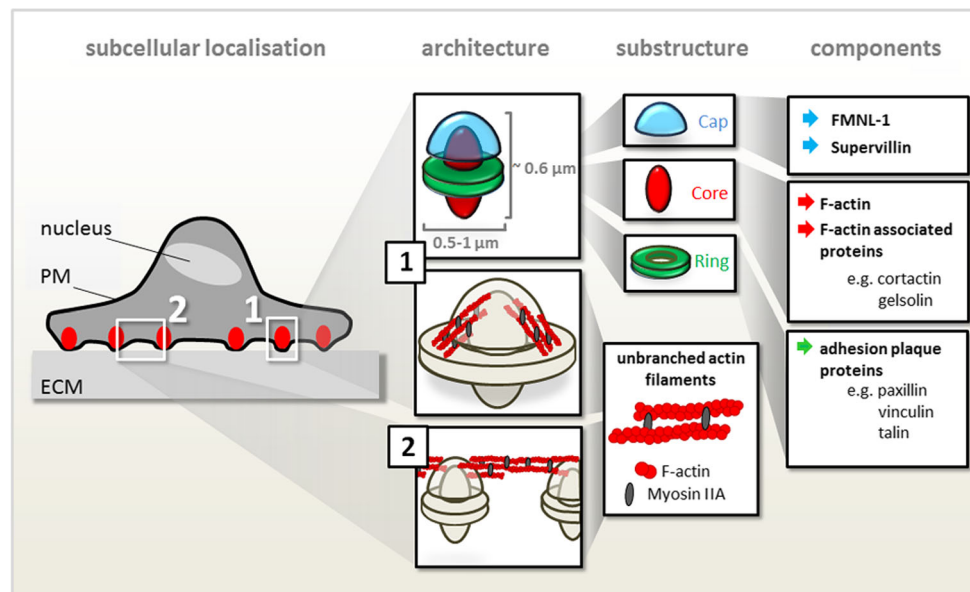


Fig. 1 Podosome architecture and composition. *Subcellular localisation*: Model of macrophage seeded on 2D matrix. Nucleus, plasma membrane, (PM) and extracellular matrix (ECM) are indicated. Note numerous dot-like podosomes at the substrate-contacting cell side (red). White boxes indicate regions shown in greater detail in further

panels. *Architecture and substructure*: (1) Model of podosome substructures (*core*, *ring*, *cap*), as well as lateral unbranched actomyosin filaments, and (2) acto-myosin cables connecting individual podosomes. *Components*: typical components of podosome *cap*, *core*, and *ring* structures are indicated

as well as several other cell types. Invadopodia, on the other hand, play a more pathophysiological role, as they have been linked to invasion of several cancer cell types, including carcinoma [11] and melanoma cells [12]. Invadosomes of v-Src transformed fibroblasts combine features of both podosomes and invadopodia [3]. They were the first discovered organelles of the invadosome group [13] and are often addressed as podosomes in the literature.

Podosomes and invadopodia share many features such as key molecular components, regulatory aspects including actin turnover, and the influence of intracellular trafficking, as well as functional properties, in particular the ability to degrade extracellular matrix material. However, they also differ in several important regards such as architecture and life time, with invadopodia showing pronounced longitudinal, protrusive growth of several μm and persistence for up to >1 h, which is in clear contrast to the smaller ($0.5\text{--}1$ μm) and more transient (~ 10 min) podosomes [7, 14, 15].

Podosomes are particularly abundant in cells of the monocytic lineage such as monocytes [16], macrophages [5], dendritic cells [6] and osteoclasts [7] (Fig. 2). These cells form podosomes in high numbers, ranging from several dozen to hundreds per cell. Podosomes thus constitute a major part of the monocytic actin cytoskeleton. Moreover, monocytic cells form podosomes constitutively (see also paragraph on macrophages and dendritic cells

below), which points to the relative importance of podosomes for these cells. At the same time, these aspects make monocytic cells excellent models to study podosome functions and properties.

In monocytic cells, podosomes enable such diverse functions as rigidity sensing [17], bone resorption, [7] and antigen sampling [18], among others. This review is particularly focused on the structure, regulation, and functions of podosomes in macrophages, dendritic cells, and osteoclasts. For further reading on other aspects of invadosome research, we would like to refer to a variety of articles, including comprehensive overviews on both podosomes and invadopodia [3, 4, 19] literature on cytoskeletal regulation [20, 21] and matrix degradation by Invadosomes [14, 22], as well as a historical perspective on invadosome research [23].

Complex clusters: podosome structure and components

Podosomes are characterized by their typical morphology and architecture. They present as dot-like, actin-rich structures at the substrate-attached cell side, with a diameter of $0.5\text{--}1$ μm , and a height of ~ 0.6 μm [24, 25]. Their F-actin core, which also contains other actin-associated proteins such as cortactin [26] or gelsolin [27], is surrounded by adhesion plaque proteins such as paxillin, vinculin, or talin [28, 29]. In immunofluorescence micrographs, these plaque proteins form a ring structure that surrounds the podosome core [30]. This classical view of a

bipartite podosome structure, consisting of core and ring, continues to be useful for the detection and initial identification of podosomes in various cell types (Fig. 1). However, use of super resolution microscopy showed that the apparently continuous ring is an artifact of fluorescence microscopy, and that plaque proteins form several discrete clusters around the core structure [31–33].

Further studies revealed that podosome architecture is indeed far more complex than initially perceived: the actin core, which is characterized by Arp2/3 complex generated, and thus likely branched, networks [9, 34, 35] is surrounded by a shell of unbranched filaments that connect the top of the podosome to the ventral plasma membrane [7, 36, 37]. An additional array of unbranched actin cables connects individual podosomes and thus helps to organize them into a higher-ordered group (Fig. 1). The contractile nature of these cables [38] is probably also instrumental in generating the typical regular pattern of podosome groups [3].

Moreover, the formin FMNL1 [39] and the villin family member supravillin [38] have been shown to form a cap

structure on top of the podosome core. This structure may serve as a hub for incoming vesicles or as a regulator of podosome growth or podosome-associated contractility [38] (Fig. 1). Consistent with this, myosin IIA, one of the main contractility-inducing proteins at podosomes [40, 41], has been shown to surround the podosome core [38], in addition to its localization to podosome-connecting cables (Fig. 1).

Podosomes are adhesive structures that establish close contact between the cell and the underlying substratum [42]. This cell–matrix linkage is achieved through transmembrane proteins that bind ECM components, notably integrins containing $\beta 1$ or $\beta 3$ subunits [29, 43], as well as the hyaluronan receptor CD44 [44] (see also paragraph on cell–substrate adhesion below).

The prototype: podosomes in macrophages and dendritic cells

Many aspects of podosome biology have been uncovered in monocytic cells, and particularly in macrophages and

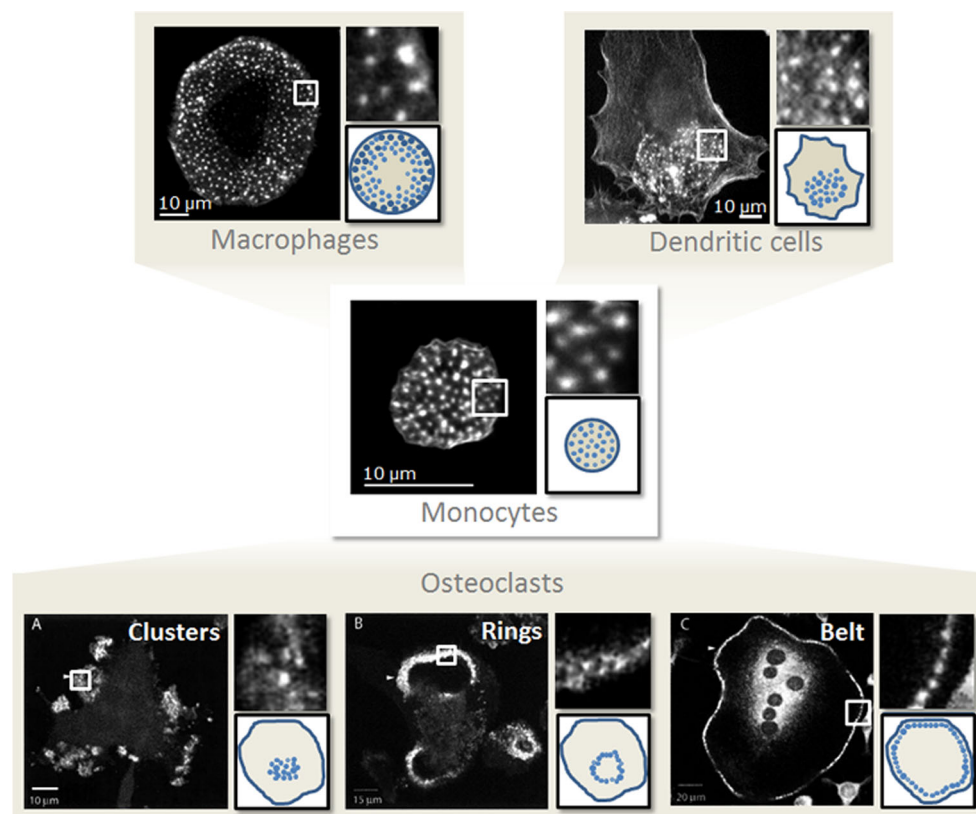


Fig. 2 Podosomes in monocytic cells. *Middle panel:* blood-derived monocyte, fixed 6 h post seeding; *upper panel:* monocyte derived macrophage and immature dendritic cell; *lower panel:* osteoclasts showing various podosomes arrangements, including clusters, rings and belts. Panels show fluorescence micrographs of cells fixed and stained for F-actin. *White boxes* indicate regions of detail images shown in *upper right sides*. Individual podosomes are visible as

F-actin-rich dots. Small insets on *lower right sides* show typical size and arrangements of podosomes in the depicted cell types. Note especially the presence of larger precursor podosomes at the cell periphery of macrophages and the various podosome superstructures in osteoclasts. *Scale bars* 10 μm . Dendritic cell image taken from [17], osteoclast images taken from [7], respectively, and reprinted with permission

dendritic cells (for osteoclast podosomes, see paragraph below). These cells to form podosomes constitutively and in high numbers, which makes them accessible for experimental intervention and statistical analysis [45, 46]. Podosomes have been detected in primary human monocytes [16, 30, 47] (Fig. 2) and primary human macrophages [5, 48] (Fig. 2), in the PMA-differentiated human monocytic cell line THP-1 [49, 50], as well as in primary human [6, 51] and murine [52] (Fig. 2) dendritic cells.

Basic principles of podosome regulation that have been discovered in macrophages and dendritic cells include the dependence on WASP- [5, 6] and Arp2/3 complex-driven [34] actin polymerization, the influence of microtubule-based transport processes [16, 40, 53, 54] and functions such as matrix degradation [55], rigidity sensing [25], topography sensing [32] and antigen sampling [18, 56] (see paragraphs on intracellular trafficking and on podosome functions below). Further important aspects include the discovery of podosome dynamics, comprising de novo formation, fusion, fission, and dissolution [38, 40, 57] and the identification of the podosome proteome, which is in the range of ~ 200 proteins [58], comparable to that of focal adhesions [2] or invadopodia [59]. Moreover, macrophages enabled the discovery of podosomes in a 3D context [60], and also the link to potentially podosome-based diseases [5, 61] (see paragraphs on podosomes in 3D environments and on podosome-associated diseases below).

Monocytic cells form podosomes constitutively. However, this ability is restricted to certain subsets of these cells. For example, it has been shown that M2 macrophages form numerous podosomes, while M1 cells are mostly unable to do so [47]. Along similar lines, only immature dendritic cells form podosomes [62]. It is noteworthy that in both cases, the ability to form podosomes is linked to an integrin-dependent migratory phenotype. It should also be mentioned that insufficient transfection rates of primary macrophages and dendritic cells have previously hindered experiments using siRNA or vector-based constructs. However, the introduction of new transfection technologies [40, 53] has helped to overcome these difficulties.

Adhesion versus degradation: podosomes in osteoclasts

Osteoclasts are unique among monocytic cells, as they form a variety of podosome-based superstructures that also exhibit extensive dynamics. Osteoclasts seeded on glass or other comparably smooth surfaces form different podosome arrangements, including clusters, expanding rings, and stable belts at the cell periphery [63] (Fig. 2).

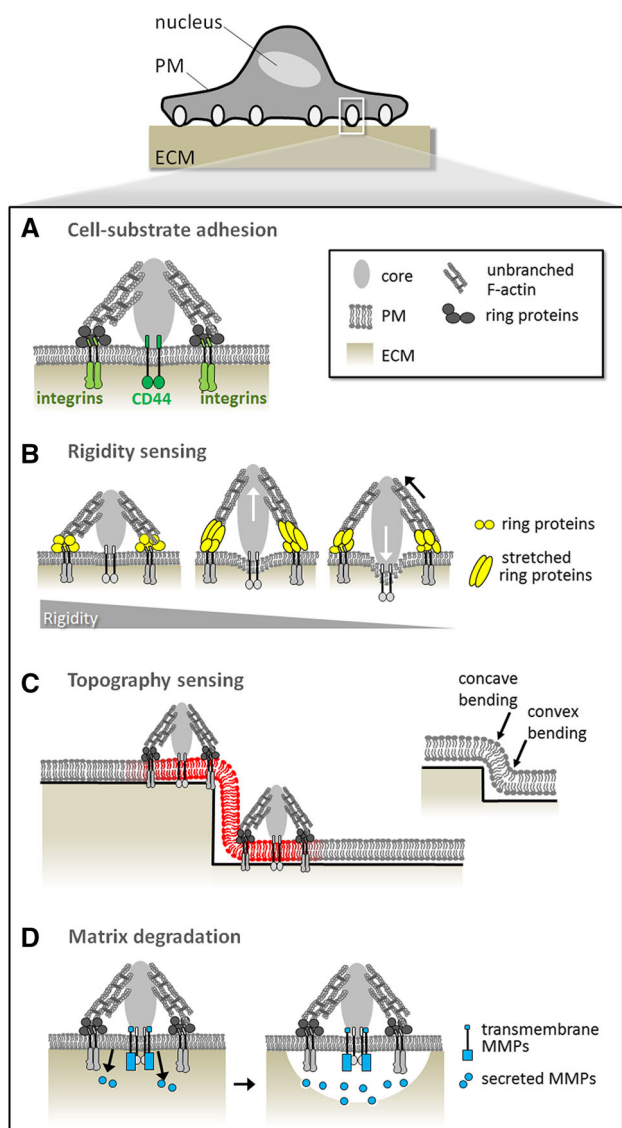
The operative podosome-based structure of osteoclasts, however, is the sealing zone (SZ). Through SZ-mediated attachment to the bone surface, osteoclasts form a closed

environment, the so-called resorption lacuna, into which protons and lytic enzymes can be secreted [64], thus enabling bone remodeling. Interestingly, SZs display an altered podosome architecture, as they consist of tightly packed podosome cores [44], while the plaque proteins of the ring structure are recruited to the inner and outer rims of the SZ [63]. The layer of unbranched cables that surrounds podosome cores is present in the form of a dense cloud of actin filaments. In fact, the term “cloud” is now more commonly used to address the layer of unbranched actin filaments around podosomes also in other cell types [65] (Fig. 1). Formation of SZ versus podosome belts is probably driven by the roughness of bone, which points to the ability of osteoclasts to sense substrate topography [66] (see paragraph on topography sensing below).

Podosomes have been detected in osteoclasts of various species, including chicken [28], human [43], rat [67], mouse [68], and rabbit [69]. Respective studies have also used osteoclast-like cells derived from the murine leukaemic monocyte macrophage cell line RAW 264.7 [70, 71].

Important milestones in podosome research using osteoclast cells include the first description of podosomes in untransformed cells, i.e. avian osteoclasts [72], the measurement of podosome lifetime (2–12 min), including the intriguing finding that podosomal actin is turned over ~ 3 times within the life span of a single podosome [7], and the finding that microtubule dynamics impact on the stability of podosome belts [73, 74]. Analysis of osteoclasts also provided the best ultrastructural description of podosomes and their substructures so far [37], and enabled the detailed analysis of protein recruitment and exit during the podosome life cycle [75]. Osteoclasts were also useful for the discovery of podosome-associated functions, such as substrate topography sensing [66] and transmigration across cell layers [65, 76]. The identification of novel RhoGTPases that impact on podosome formation or patterning [77, 78], such as RhoU [79] and RhoE [80], was also performed in osteoclasts. Finally, correlated defects in podosome/SZ formation and bone resorption point to links with respective pathologies [27, 81] (see paragraph on podosome-associated diseases below).

A major unsolved question concerns the non-lytic nature of the osteoclast SZ. To avoid leakage of the resorption lacuna, SZ have to closely adhere to the substratum. Matrix-lytic processes at the SZ itself would undermine this function. It could be imagined that the different architecture of SZ results in a failure to recruit respective proteinases. However, experimental proof for such a scenario is lacking. Still, this observation highlights the fact that podosome-based matrix degradation is a secondary phenomenon that can, in principle, be separated from podosome formation and turnover (see also paragraph on matrix degradation below).



◀**Fig. 3** Podosome functions, part I. Model of macrophage seeded on 2D matrix. Nucleus, plasma membrane (PM) and extracellular matrix (ECM) are indicated. (a–d) Established functions of podosomes. Legend in upper right indicates further details. **a Cell-substrate adhesion:** Adhesion to substratum is established by podosome-localized transmembrane proteins such as integrins (*light green*) and CD44 (*dark green*) that connect extracellular matrix components and intracellular cytoskeleton. (**b, c**) Sensing of substrate properties. **b Rigidity sensing:** Podosomes exert forces on the plasma membrane by growth of the actin-rich core structure (*white arrows*). This generates a counterforce (*black arrow*) at the lateral cables and a stretching of tension-sensitive ring proteins such as vinculin or talin (*yellow*). Depending on the more or less pronounced rigidity of the substrate (*gray slope*), podosomes are thus able to protrude to lesser or higher degree into the substrate. Note that all forces are generated on all substrate rigidities, but are only illustrated once each. **c Topography sensing:** Podosomes are formed especially at topographical discontinuities in the substrate. Increased membrane curvature (*red*), possibly leading to recruitment of curvature-sensing proteins, might help to locally attract and restrict actin polymerization of podosome cores at these sites. Sites of concave and convex membrane bending are illustrated. **d Matrix degradation:** Podosomes recruit matrix-lytic enzymes, particularly matrix metalloproteinases (MMPs). Enrichment of membrane-associated MMPs (*blue boxes*) at the ventral membrane of podosomes or secretion of soluble enzymes (*blue dots*) leads to localized degradation of matrix material at podosomes (*white halo*)

Cell-substrate adhesion

Contact with an underlying substratum is an essential prerequisite for podosome formation. Accordingly, podosomes are only formed at the substrate-contacting side of the cell (Fig. 3). This observation implicated podosomes early on as sites of matrix adhesion. Indeed, podosomes are enriched in cell–matrix receptors such as integrins [28, 82, 83] or the hyaluronan receptor CD44 [44] (Fig. 3a). Combined with the finding that podosomes establish close contact with the substratum, as visualized by total internal reflection microscopy (TIRF) [42], podosomes are thus clearly sites of close adhesion to the respective surface. Interestingly, while adhesion-permissive substrate is a prerequisite for podosome formation, the specific chemical nature of the substrate is not decisive [17], indicating that podosomes are very adaptable cell–matrix structures. Moreover, podosomes are able to gather information about the substrate composition by engagement of specific matrix ligands such as CD44 for hyaluronan [44], or integrins containing different subunits such as $\beta 2$ [62] or $\beta 3$ [44], that mediate binding to matrix components such as fibronectin or vitronectin, respectively, and result in the activation of different signaling cascades within cells [84].

Substrate sensing

In addition to sensing matrix composition, podosomes also gather information about specific physical properties of the

Multipurpose organelles: the growing list of podosome functions

Podosomes combine several abilities that enable them to establish and control the interaction of cells with their environment. Some of these functions, such as adhesion or substrate sensing, are closely linked. Others, such as adhesion and matrix degradation appear to be counterintuitive at first glance and have to be closely controlled in a spatiotemporal context. Also, not all of the known podosome functions have been studied to the same degree. Currently, adhesion, substrate sensing, and matrix degradation are widely accepted and intensely studied functions. Other aspects, including stabilization of cell protrusions, support of cell transmigration and antigen sampling, still require more in-depth exploration.

substratum such as rigidity and topography. Work in fibroblasts had already revealed that podosomes function as mechanotransduction devices [85, 86]. A pioneering study using atomic force microscopy further showed that podosomes of human macrophages undergo two internal cycles of stiffness that are based on actin polymerization and myosin II contractility [25]. Studies using human dendritic cells expanded on these findings by demonstrating that also the podosome ring structure undergoes oscillations. Importantly, it was also shown that ring and core oscillations are coupled, and that growth of the actin core drives the recruitment of tension-sensitive ring components such as vinculin and zyxin [32].

The ability for mechano/rigidity sensing is probably based on the specific architecture of podosomes, which combines a protrusive actin network in the core, an adhesive module in the ring, and a coupling of both systems by contractile actomyosin cables that connect the top of the podosome with the adhesive ring [75, 87] (Fig. 1). Rigidity sensing of the substratum could involve forces generated between the growing actin network of the core and the more stable layer of unbranched actin filaments that surrounds the core [75, 87] (Fig. 3b). As myosin IIA also localizes to these cables [38], a contribution of actomyosin contractility to this process is highly likely. It may also involve stretching of mechanosensitive proteins such as talin, vinculin, or kindlin in the ring structure of podosomes [32, 88]. The latter possibility has not been explored for podosomes yet, but stretching of talin in focal adhesions of fibroblasts has been demonstrated [89, 90], and depletion of kindlin-3 results in disorganized ring structures of macrophage podosomes [91].

Collectively, this leads to a model in which growth of the core by actin polymerization exerts a force on the plasma membrane, generating a counterforce on the lateral actomyosin cables, which leads to recruitment of tension-sensitive ring components such as vinculin and zyxin [87]. Depending on the stiffness of the matrix, the actual extent of these forces will lead to according levels of tension-sensitive components, thereby transducing the physical property of the substratum into biochemical signals at podosomes, which can then be further transmitted within the cell (Fig. 3b).

Another podosome-relevant parameter of the substratum is topography. Accordingly, osteoclasts have been shown to form small, unstable sealing zone-like structures on smooth surfaces such as glass, in contrast to larger and more stable structures on rough surfaces such as calcite or bone [92]. Sealing zone-like structures are able to detect variations in surface topography that are larger than 3 μm , i.e. larger than the size of an individual podosome [66]. Together with the observation that local inhibition of sealing zone expansion by topographical obstacles can be overcome by pulling

forces generated by adjacent parts of the sealing zone, this seems to indicate that topography sensing is performed on the level of podosome groups or superstructures.

Work using human dendritic cells, however, showed that topography also works on the level of single podosomes. Dendritic cells seeded on 3D micropatterned surfaces align their podosomes along the edges of these surfaces (Fig. 3c). Interestingly, podosomes formed at these edges are more stable, while the total number of podosomes per cell is unaltered [17]. The link between substrate topography and podosome formation is probably established by alterations in membrane curvature at the edges of the 3D substrate. Local membrane curvature is known to attract specific lipids such as cholesterol and also curvature-sensing proteins [93]. This probably leads to the generation of microdomains that allow localized RhoGTPase signaling and actin nucleation [17]. In line with these considerations, the membrane curvature protein FBP17 has been detected at podosomes of human macrophages [94]. As podosomes are formed at both sides of the curvature, i.e. at sites of concave and convex membrane bending (Fig. 3c), it is likely that podosomes are formed adjacent to, but not directly at the site of altered membrane curvature. Membrane bending thus seems to both attract and restrict podosome formation. The molecular pathways that trigger this phenomenon still have to be elucidated.

Collectively, podosomes have been revealed are organelles that are sensitive to substrate properties such as rigidity or topography. It is also noteworthy that matrix topography acts upstream of podosome formation, by determining the sites of podosome emergence and influencing podosome stability, whereas sensing of matrix rigidity is performed downstream of podosome formation. Podosomes thus function as active sensors of substrate rigidity, but as more passive reporters of substrate topography.

Matrix degradation

Degradation of extracellular matrix material is one of the hallmarks of podosomes [24]. It is also an important criterion to distinguish podosomes from other actin-rich structures. In contrast to the related invadopodia, which are highly protrusive and can persist for more than 1 h [15], podosomes are only protrusive to a minor degree and are turned over within minutes [7, 14]. Podosome-mediated substrate degradation is therefore rather shallow. However, the high number of podosomes per cell, which is especially pronounced in primary macrophages [30], allows cells to quickly degrade a comparatively large area.

Podosomes degrade ECM by recruiting matrix-lytic enzymes, particularly metalloproteinases such as matrix metalloproteinases (MMPs) or ADAMs (a disintegrin and metalloproteinase) (Fig. 3d). These proteases are

subsequently secreted at the ventral plasma membrane or, in case of membrane-associated enzymes, exposed on the ventral surface of podosomes. Considering that monocytic podosomes are the prototype organelles of lytic adhesions, surprisingly little is known about the involvement of matrix metalloproteinases and other matrix-lytic enzymes especially in these cells. Indeed, most of the current knowledge on matrix-degrading proteinases at podosomes has been gained using other cell types such as v-Src-transformed fibroblasts or endothelial cells [3, 4] (see also paragraph on podosomes in 3D environments below).

In case of monocytic cells, the membrane-bound MMP isoform MT1-MMP is the best-studied podosome-associated proteinase. It has been detected at podosomes in primary rabbit osteoclasts [69], at vesicles that contact podosomes in primary human macrophages [53], and at podosomes of RAW 264.7 macrophages [95]. Recruitment of MT1-MMP to podosomes involves microtubule-dependent transport of vesicles by kinesin-1 and -2 motor proteins [53] and RabGTPases [104] (see also paragraph on microtubule-dependent transport below).

As only the secondary enrichment of MT1-MMP at pre-existing podosomes enables local matrix degradation, matrix lysis can be viewed as an acquired ability of podosomes, and not an intrinsic one, i.e. not performed by factors that are essential podosome components. Accordingly, podosome formation and dynamics can proceed in the absence of podosome-associated matrix degradation [96]. Still, feedback loops between both phenomena have been proposed [14], as, for example, inhibition of MMPs can lead to increased life spans of osteoclast podosomes [97] and knockdown of MT1-MMP limits the protrusion of dendritic cell podosomes on porous filters [18]. However, it is currently unclear whether this reflects actual feedback from the degradation process or whether it is based on proteolytic processing of podosome components. Still, as cleaved matrix is also internalized by cells, this process could also help cells to gather additional information about their environment.

Protrusion stabilization

Podosomes are often formed in protruding parts of the cell such as the leading edge of migrating cells. This is especially evident in dendritic cells, which form podosomes only during their immature, i.e. integrin-dependent migratory, stage, but not in their mature form [62]. This led to the hypothesis that podosomes are involved in directional migration of cells. Formal proof for such a function is still missing, but it could be envisioned that podosomes help to stabilize otherwise transient protrusions and may thus

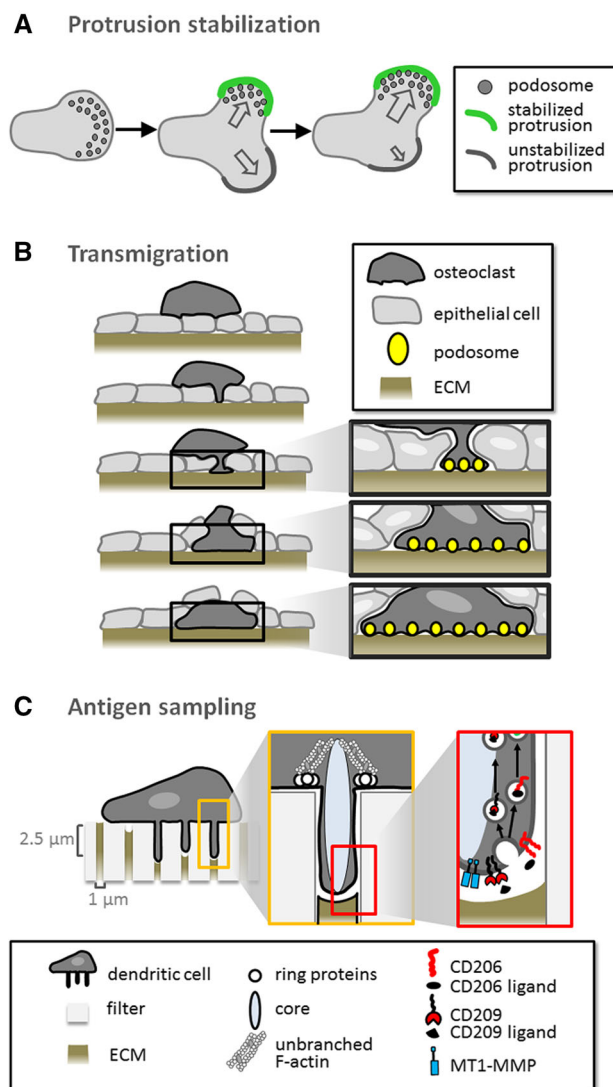


Fig. 4 Podosome functions, part II. Podosome functions needing more in-depth investigation. **a** *Protrusion stabilization*: Podosomes (gray dots) are especially formed at leading edges of migrating cells. Recruitment of podosomes to newly developed protrusions (arrows) might stabilize them (green) and thus favor a respective direction of migration (large arrow). Protrusions that fail to recruit podosomes might be more unstable (gray) and would thus contribute less to the direction of migration (small arrow). **b** *Transmigration*: Osteoclast (dark gray) seeded on a layer of epithelial cells (light gray) can transmigrate by formation of protrusions. Contact with the underlying substratum (fawn) enables the formation of podosomes (yellow) anchoring the cell front. Final transmigration of the cell body is probably supported by actomyosin contractility. Black boxes in overview images on left indicate area of detail images on right. **c** *Antigen sampling*: Dendritic cells (gray) seeded on porous filters (light blue) form protrusions that are enriched in typical podosome components. These protrusions contain MT1-MMP and can degrade extracellular matrix (fawn). Moreover, the protrusions also contain antigen receptors such as CD206 and CD209 and are sites of respective ligand uptake. Black boxes in left and middle images indicate areas of adjacent detail images

contribute to efficient, and possibly also directional, cell migration [3, 62, 99] (Fig. 4a).

Transmigration

A combination of two functions, adhesion and protrusion stabilization, apparently enables cell translocation across layers of other cell types. RAW cell-derived osteoclasts have been shown to extend their protruding front through layers of osteoblasts, endothelial cells or adipocytes in vitro [65, 71]. Once the cell front contacts the underlying substratum, it is anchored by formation of podosomes, which enables the transmigration of the cell body, most probably through actomyosin-based contractility (Fig. 4b). Use of pharmacological inhibitors showed that osteoclast transmigration depends on the activity of matrix metalloproteinases, Src, and Rho-GTPases, all of which are critical regulators or effectors of podosomes [65].

Antigen sampling

On filters containing pores of 1 μm size, dendritic cells form actin-rich structures that protrude into the pores. These protrusions contain typical podosome components such as vinculin, paxillin, and talin that are arranged in a ring around the pore (Fig. 4c). They are also positive for MT1-MMP [56], showing that these structures are potentially degradative. Interestingly, these protrusions are also sites of endocytosis, as shown by uptake of gold particles [18]. Moreover, they contain pattern recognition receptors such as the mannose receptor CD206 or the lectin family receptor CD209 [56]. Consistently, these protrusions are able to internalize ovalbumin [56], a ligand for CD206 [100], and also the HIV1 glycoprotein gp120 [56], a ligand for CD209 [101] (Fig. 4c). Collectively, these data show that podosome-like protrusions of dendritic cells are sites of receptor-mediated uptake of antigen. As both podosome formation and antigen sampling are restricted to the immature stage of dendritic cells, the temporal correlation appears to be consistent. To confirm that these structures are indeed derived from podosomes, it would be important to visualize their development in live cell imaging.

Spot-on delivery: microtubule-dependent transport to podosomes

The fine-tuned turnover of actin filaments and the concerted activity of a plethora of actin-associated proteins are essential for the formation and regulation of podosomes. In addition, microtubule-dependent transport has emerged as a major factor that impacts on many levels of podosome turnover and function. These aspects have been studied

particularly in macrophages and osteoclasts. Microtubules influence most aspects of podosome dynamics, as they are involved in the formation of podosomes [16], the fusion and fission of individual podosomes [40, 57], and also in their dissolution [40]. In osteoclasts, microtubules have been shown to influence the subcellular positioning of podosomes [73, 77, 102], as well as the stability of podosome superstructures such as rings and belts, and also the integrity of the sealing zone [63]. Moreover, microtubules are involved in the functional aspects of podosomes, particularly in their matrix-degrading ability [53, 54, 103].

Microtubules act as a long-range transport system that delivers components, regulators, and effectors to podosomes or to sites of podosome formation [3]. In this context, microtubule-dependent transport can be viewed as a tripartite system, where specific cargo (vesicles or molecules) is transported by motors (kinesin and dynein motor proteins) along specialized tracks (microtubules) (Fig. 5). Experimental evidence suggests that molecular alterations of all three parts of this system are possible, to ensure spatiotemporally correct delivery of specific cargo to podosomes during all stages of their life cycle.

Motors

Motor proteins of the kinesin family have emerged as major regulators of podosome-directed transport (Fig. 5). Kinesin-1 and kinesin-2 are both involved in the transport of MT1-MMP [53], while KIF1C, a kinesin-3 motor, regulates podosome fission and dissolution, through delivery of as yet unidentified cargo [40]. KIF-9 is the motor responsible for transporting reggie-1 positive vesicles. Apparently, it also transports at least one other component that also impacts on podosome formation [54].

Cargo

The influence of microtubule-dependent transport events is most evident in the case of proteins that regulates specific functions of podosomes. Accordingly, the vesicle regulator reggie-1 has been shown to influence matrix degradation by macrophages podosomes [54]. MT1-MMP, a key enzyme in matrix degradation of podosomes, is transported to podosomes [53] in vesicles whose trafficking is regulated by the RabGTPases Rab5a, Rab8a, and Rab14 [104] (Fig. 5).

Tracks

Microtubules are not passive tracks for cargo transport. Indeed, their dynamic behavior, their transient contact with podosomes and also their secondary modification can influence several aspects of podosome regulation and function.

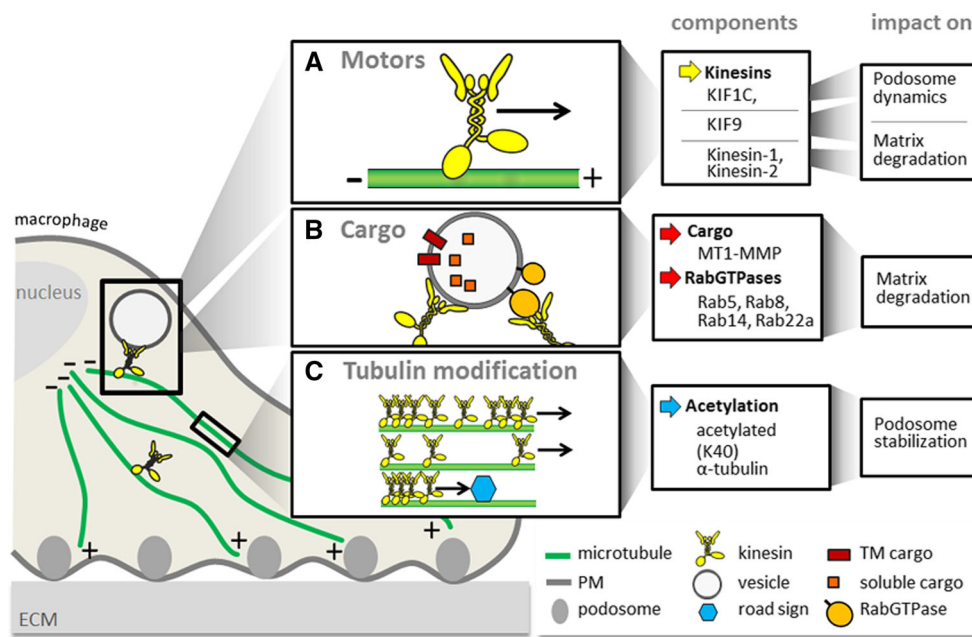


Fig. 5 Microtubule-dependent transport to podosomes. Microtubules (green) contact podosomes (gray dots) with their plus ends (indicated by “+”). **a** Plus end-directed motor proteins of the kinesin family (yellow) transport, **b** cargo in the form of vesicles that contain transmembrane (TM) or soluble proteins and are regulated by RabGTPases. **c** Transport along microtubules can be influenced by

secondary modifications, e.g. acetylation of α -tubulin at the K40 residue, that may act as “road signs”, influencing attachment, run length, or velocity of motors. Components of the respective groups with demonstrated impact on podosome dynamics and formation or podosomal matrix degradation are indicated

1. Microtubules contact podosomes via their plus-tips [40]. Proteins that capture incoming plus ends at podosomes include cortactin, which interacts with the plus-tip protein EB1 [74], and possibly myosin X, which binds to both actin and tubulin [105]. Accordingly, depletion of either EB1, cortactin or myosin 9b leads to impaired positioning of podosome belts and defects in sealing zone formation and bone resorption in murine osteoclasts [74, 102].
2. The efficiency of microtubule-dependent transport probably also depends on regulatory pathways that influence microtubule stability. Accordingly, reduced activity of histone deacetylase HDAC-6, leading to increased acetylation of α -tubulin and thus to enhanced microtubule stabilization, is important for the stability of podosome belts and sealing zones in osteoclasts [73, 106]. In this context, HDAC-6 activity is regulated by pathways involving mDia2 and RhoA [73], as well as c-Cbl and Cbl-b adaptor proteins [106]. Further regulators that impact on RhoA-dependent regulation of microtubule stability and SZ formation include Pyk2 [81] and myosin 9b [107].
3. Secondary modifications of tubulin have been speculated to act as “roadsigns” that hinder or enable the movement of specific motor proteins, and to thus impact on cargo delivery [108]. Although this

hypothesis has been under debate, genetically engineered tubulins bearing specific posttranslational modifications have been recently shown to influence motor protein properties [109]. In line with these analyses, acetylation of α -tubulin has been demonstrated to influence the velocity, directionality, and run length of KIF1C-positive vesicles and to thus impact on podosome formation in macrophages [110] (Fig. 5).

Lost in space? Podosome formation in 3D environments

Most of the current knowledge about podosomes has been gained by the study of cells in 2D culture. Considering that 2D cell-substrate interactions also exist in the body, for example monocytes adhering to blood vessel endothelium or osteoclasts to bone surfaces, formation of similar structures in a 2D in vivo context is very likely. However, cells in vivo are often embedded in a 3D environment, such as the fibrillar network of the extracellular matrix. Increased effort is therefore being made to study formation and regulation of podosomes also in 3D systems.

The molecular composition of 3D matrices can be heterogeneous, comparable to 2D substrates. However, also additional variables come into play, such as microstructure or

viscoelastic properties of the matrix. Both factors are based on the highly variable nature of intra and intermolecular connections of matrix molecules and are crucial for the migration mode that cells adopt [111, 112]. For example, low matrix connectivity will enable cells to squeeze through matrix gaps, thus resulting in amoeboid migration, which does not require matrix lysis. On the other end of the spectrum is mesenchymal migration, in which cells locally degrade matrix material and are thus able to invade also through highly connective matrix. Both forms are not mutually exclusive, and cells are probably able to switch between migration modes as necessary. Factors influencing this switch are mainly pore size of the matrix and the deformability of the cell nucleus [111–114]. Moreover, acquisition of a mesenchymal migration mode by macrophages has been shown to involve downregulation of Rho/ROCK signaling by the cyclin-dependent kinase p27^{kip1} [115].

Macrophages can adopt both amoeboid and mesenchymal migration modes [48, 60, 104, 116], which makes them unique among leukocytes, which usually only use amoeboid migration [47]. Macrophages seeded in gelled or fibrillar collagen I matrix have been shown to migrate mesenchymally and from numerous long protrusions [60, 104]. Strikingly, the tips of these protrusions often contain F-actin accumulations and are also enriched in proteins that are typically localized to podosomes, including F-actin binding proteins such as cortactin and gelsolin, adhesion plaque proteins such as vinculin and talin, and transmembrane proteins such as $\beta 1$ integrin and CD44. Moreover, these F-actin dots show also a podosome-typical enrichment of phosphotyrosine residues [117], they recruit the metalloproteinase MT1-MMP [104], various cathepsin proteinases [118], and are also sites of local ECM degradation [104, 117]. Despite differences in size and architecture, these structures are thus the morphological and functional equivalents of classical 2D podosomes and have accordingly been termed “3D podosomes” [112]. The life time of 3D podosomes is similar to that of their 2D counterparts (4.9 ± 0.5 min) [117]. However, they show decreased numbers per cell (2–9 vs. >100) and increased size (~ 5 μm diameter vs. 0.5–1 μm), compared to 2D podosomes.

3D podosomes recruit MT1-MMP and colocalize with degraded matrix material [104, 117]. They are thus clearly degradative organelles. The presence of cell–matrix contact proteins such as $\beta 1$ integrin and CD44 [117] also implies a function in cell–matrix adhesion. Currently, it is unclear whether they also function as substrate sensors. As the specific architecture of 2D podosomes appears to be essential for this function (see paragraph on podosome functions), this appears to be less likely.

Interestingly, a recent study revealed that the ability to form numerous podosomes in 2D and the extent of cell

invasion in 3D are correlated. Accordingly, blood-derived, and also M2-stimulated macrophages, both of which contain numerous podosomes, are able to mesenchymally invade into dense matrices such as Matrigel. By contrast, M1-stimulated cells and resident macrophages harvested from the peritoneal cavity of mice were unable to do so [47]. It will be highly interesting to test these macrophage subtypes for formation of 3D podosomes. Moreover, further studies using dendritic cells should help to clarify whether formation of 3D podosomes is a more widespread phenomenon.

Dangerous defects: Podosome-associated diseases

Absent or impaired formation of podosomes has been linked to a variety of diseases. Wiskott–Aldrich Syndrome (WAS), a multisystemic disorder associated with immune defects and based on mutations in the *WASP* gene, is a prominent example. Accordingly, macrophages and dendritic cells from WAS patients are unable to form podosomes in 2D cell culture [5, 6]. Although it has not been tested yet, this probably also translates to an inability of cells to form 3D podosomes, and impairment of one or both manifestations could explain the observed defect in WAS immune cell migration [119, 120]. Moreover, the newly discovered role of dendritic podosomes in antigen sampling [18, 56] (see also paragraph on podosome functions above) might contribute to the observed immune defects in WAS.

A more recently established link to a disease concerns PAPA (pyogenic sterile arthritis, pyoderma gangrenosum, and acne) syndrome, which is based on mutations in the gene for the cytoskeletal adaptor PSTPIP1. An R405C amino acid exchange, for example, leads to reduced podosome formation, enhanced numbers of filopodia, and matrix degradation in human macrophages [61]. Interestingly, WASP is also involved in this phenomenon, as the R405C exchange results in impaired WASP binding of PSTPIP1 [61]. It has to be noted that, for both WAS and PAPA syndrome, the activity of WASP or PSTPIP1 is not restricted to podosomes or filopodia, but is likely to affect the whole cytoskeleton. Abnormalities in podosome formation or function are thus clearly correlated with both symptoms, but might not be causative.

Defects in osteoclast SZ formation or dynamics are likely to lead to altered bone resorption, and ultimately to osteoporosis or -petrosis. Accordingly, WASP deficiency results in defective SZ formation and abnormal bone resorption in mice [121]. Similarly, murine knockout of Src or the Src family kinase Hck both lead to defective SZ formation and osteopetrosis [122, 123]. Knockout of gelsolin, which forms a complex with Src and PI3K that regulates SZ formation, leads to a similar phenotype [27].

Also, deficiency of the podosome ring component kindlin-3 has been shown to result in impaired integrin recruitment, defective SZ formation and osteopetrosis in mice [124]. Moreover, patients suffering on the kindlin-3-based leukocyte adhesion deficiency (LAD)-III, also exhibit an osteopetrotic phenotype [92].

Finally, even per se undiseased macrophages can promote pathological states, such as invasion of tumor cells. Indeed, the number of tumor-associated macrophages (TAMs) correlates with poor patient prognosis [125, 126]. This seems to be based on the ability of macrophages to stimulate invadopodia formation in cancer cells through a paracrine loop that involves epidermal growth factor (EGF) [55]. In addition, TAMs might also directly contribute to tumor cell migration by degrading the tumor-associated extracellular matrix, which would facilitate tumor growth and dissemination of metastasizing cells [127]. In 3D cell culture, macrophage-stimulated infiltration of tumor cells has been shown to depend on MMP-dependent matrix degradation by macrophages [128]. A participation of 3D podosomes in this process is likely, but formally unproven yet.

Conclusions

The growing list of podosome functions highlights these structures as multipurpose organelles of monocytic cells. Podosomes not only enable dynamic interactions of cells with their surroundings, but also gather information about the pericellular environment, and are actively involved in its reshaping. Several of these functions are based on intrinsic properties such as the particular architecture of podosomes. For example, rigidity sensing of the substratum probably involves forces generated between the growing actin network of the core and the more stable layer of unbranched actin filaments that surrounds the core (Fig. 3b) [75, 87]. In contrast, other functions such as degradation of matrix material requires the recruitment of additional factors such as matrix-lytic enzymes that have to be delivered by microtubule-dependent transport [53, 54]. Moreover, seemingly contradictory abilities of podosomes, such as substrate adhesion and matrix degradation, indicate that the podosome functions have to be exquisitely orchestrated in a spatiotemporal context. This raises intriguing questions, such as whether all podosomes in a given cell might be degradative at the same time, or how the adhesive and non-lytic nature of osteoclast sealing zones is achieved. Together with the increasingly detailed analysis of podosome formation also in 3D environments and the link to potentially podosome-associated diseases, monocytic podosomes will continue to attract scientific curiosity and experimental efforts for the foreseeable future.

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