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Cutting Edge

Surface wound healing: a new, general function of eukaryotic cells

J. Meldolesi *

DIBIT, Department of Neuroscience and Center of Excellence in Physiopathology of Cell Differentiation, Vita-Salute San Raffaele University and IRCCS San Raffaele, Milan, Italy

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Abstract

The ability to repair surface wounds is a property, necessary for long-term survival, expressed to various extents by all eukaryotic cell types except erythrocytes. The process is based on the rapid Ca^{2+}-induced exocytosis of various types of specific organelles, such as lysosomes and enlargeosomes, that decreases surface tension and makes possible the spontaneous fusion of lipid monolayers at the lesion edges. The recognized importance of the process in physiology and in several cases of pathology is discussed.

Keywords: membrane repair - membrane surface tension - exocytosis - lysosomes - enlargeosomes - calcium

Introduction

The variable phenotype of eukaryotic cells can be envisaged as the result of two specific groups of features and functions: those typical of specialization (*e.g.* regulated secretion, coordinate contraction, excitability, motility etc.) superimposed on a spectrum of functions common to almost all types of cells. Many of these common functions (*e.g.* gene expression, protein synthesis, energy transduction, cytoskeleton assembly and contraction, constitutive secretion) are known since long time. In view of the actual comprehensive information about the cell and its functions, they were considered by most scientists as fully representative of the state of non-specialized cells. The identification of new such functions was therefore not expected. Yet, during the last several years progress of technology accompanied by the wider circulation of ideas among the various

* Correspondence to: Jacopo MELDOLESI,
DIBIT, Dept. of Neuroscience and Center of Excellence in Physiopathology of Cell Differentiation,
Vita-Salute San Raffaele University and IRCCS San Raffaele, via Olgettina 58, 20132 Milano, Italy.
Tel.: +39 02 26432770, Fax: +39 02 26434813,
E-mail: meldolesi.jacopo@hsr.it

areas of biological and biomedical sciences have clarified new aspects of cell life and lead to the identification of a few general functions that had not been previously recognized. Among these is wound healing, by which lesions of the plasma membranes do not evolve always towards necrosis, as previously believed, but can be rapidly and appropriately repaired. Not surprisingly this function, which is now recognized as necessary for long-term cell survival, is especially important in a long list of conditions, physiological as well as pathological. In the present short review I will present various aspects of the wound healing process, beginning with a historical account of the development of knowledge in the field.

History

The ability to repair discontinuities of the plasma membrane was first reported in 1930 for peculiar cells of large volume. However these repair responses, defined as "reactions of superficial precipitation" [1], attracted little attention and were rapidly forgotten. Approximately 25 years ago interest about this process has begun to raise. In many laboratories, in fact, it was realized that a number of widely used experimental techniques such as cell cultures, which most often imply the application of mechanical traumatisms, especially during passages; cell punctures with microelectrodes and others, inevitably induce wounds of the cells. Additional procedures introduced in the meantime, such as fusion of cells to give chimerae; swelling and electroporation to introduce into the cells impermeant dyes, proteins or gene constructs, are also based on the generation of discontinuities of the cell surface. Most cells amputated of cytoplasmic fragments and expansions (during resuspension and dissociation), punctured, fused or electroporated remain alive and are able to proliferate. Their ability to heal wounds had therefore to be given for granted. Yet, for reasons probably dependent more on the cultural background of scientists rather than on experimental evidence, all the data were accepted by the scientific community without questions about wound healing.

A final historical example of membrane repair I want to give concerns pathology of nerve cells. The frequent survival of motor neurons after section of their axons, followed by re-innervation of muscle, is well known. Indeed, at the moment extensive studies are carried out aimed to overtake the block of regeneration occurring in lesioned axons of the central nervous system. Yet, for many years little attention was given to the processes necessary for the axons to re-acquire the continuity of their membrane, a step necessary to trigger the regenerative process. In this case, therefore, interest was focused exclusively on the final, successful outcome, and the initial repair events were given no attention.

The real breakthrough in the field occurred in the late eighties when the groups of Steinhardt and Terasaki in the U.S.A. [2, 3] have begun to investigate the series of events taking place in the urchin eggs following mechanical treatments inducing lesions up to 100 μm in diameter [4]. Most of these cells were able to repair the lesion by a process including exocytosis of large organelles, the yolk granules. At this point, and for a few years, the process was considered specific of ekinoderm eggs and of their peculiar organelles. Yolk granules, however, are peculiar only in part since they represent a specialized version of an ubiquitous organelle, the lysosome. When repair was demonstrated in widespread cell types, beginning with fibroblasts [2, 3], attention was therefore focused on lysosomes as the exocytic organelles sustaining repair [5, 6]. At the moment the scenario appears more complex than previously believed, inasmuch as the participating organelles are probably not of one type only, but of at least two and possibly more types.

Cell specificity of repair

Before discussing the mechanisms, let's consider the types of cells that are competent for the process and the conditions existing when healing takes place. As already anticipated, competence of cells seems widespread and possibly general, with the only exception of erythrocytes which, being devoid of cytoplasmic organelles, cannot undergo the coordinate fusion sequence taking place during the repair process. There is no doubt, however, that the frequency of wounds varies profoundly among cell types and that it can change depending on

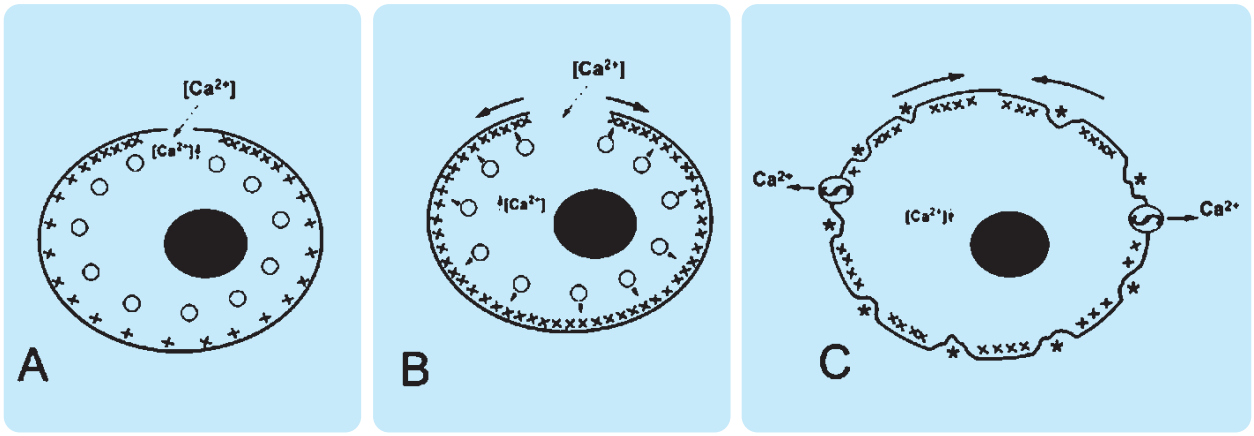

Fig. 1 The multiple exocytosis model of wound healing.
Panel A shows a cell containing many membrane delimited organelles (circles) competent for Ca^{2+}-dependent exocytosis, just wounded on its surface. Due to the strong electrochemical gradient of Ca^{2+} across the plasma membrane, the wound induces a large influx of the cation, with ensuing increase of the cytosolic Ca^{2+} concentration localized initially in the area adjacent to the wound. This, in turn, initiates locally the contraction of the sub-plasmalemmal cytoskeleton (represented by the thickening of the x-composed layer), with increase of the surface tension of the cell (Panel A). A few seconds later (Panel B) the increase in cytosolic Ca^{2+} concentration has expanded to the entire cell, with increase of the cytoskeletal contraction/surface tension, and with ensuing widening of the wound in the direction of the arrows. Concomitantly, however, the increased concentration of cytosolic Ca^{2+} induces the transfer of the exocytic organelles towards the plasma membrane (arrowheads, panel B). A few seconds later (Panel C) the organelles have undergone exocytosis (*) with ensuing enlargement of the plasma membrane. As a consequence, and in spite of the cytoskeletal contraction, the surface tension is decreased and the margins of the wound are now close to each other (arrows). A spontaneous membrane fusion, with repair of the wound, has thus become possible. Concomitantly, the cytosolic Ca^{2+} concentration decreases due to decreased and then stopped Ca^{2+} influx due to the wound repair, accompanied by the activation of the surface Ca^{2+} pumps ( ).

physiological and also pathological conditions. Most often the appearance into the cytosol of living cells in culture of tracer molecules of high molecular weight applied to the medium is interpreted as a demonstration that, in the course of an incubation, the continuity of the plasma membrane has been interrupted. More advanced procedures are however also available by which cells of living animals and also humans can be appropriately investigated. Based on the results obtained so far it can be concluded that among the cells most frequently wounded and repaired are endothelial cells, which are continuously hit by circulating cells; fibroblasts and mobile cells, which leave cytoplasmic fragments attached to their substratum; mucosal cells, especially those of the esophagus and stomach, as well as contractile fibers of cardiac and skeletal muscle [2]. Interestingly, the percentage of wounded skeletal muscle fibers varies substantially depending on the activity. It is low in resting conditions, higher during prolonged efforts, reaching values that approach 50% during races such as a marathon [2]. Also interesting is the fact that a defect in a specific exocytic type of vesicle that appears to participate in repairing seems to be responsible for the appearance of a form of genetic muscle dystrophy in which the distrophyn system is unaffected [7]. The examples given so far confirm that plasma membrane repair is necessary to survival, especially on the long run.

Mechanisms

A reason for the astonishing and long-lasting lack of attention about the membrane repair process was most likely a consequence of the wide experience of many membrane scientists in the field of phospholipid monolayers. The well known ability of the latter to seal, giving rise to continuous arti-

ficial membranes and vacuoles, able to fuse to each other and to fission into smaller membrane-bound vesicles, appeared in fact to many as a reasonable explanation of the observed membrane repair events. In other words, wound healing was considered to be a spontaneous process, taking place because of the phospholipid dynamics at the edges of the lesioned monolayer. The importance of membrane lipids is certainly fundamental for healing to take place. However, what is enough for inducing fusion of monolayers is only a condition *sine qua non* for the repairing fusion of surface membranes in living cells. A number of other processes, in equilibrium to each other, are needed to make the monolayer fusion possible.

The first such process to be considered is contraction of the cytoskeleton. On its cytosolic surface the plasmalemma is in fact coated with a layer of contractile filaments directly attached to the membrane by specific proteins (Fig. 1A). Even at rest the interaction with the cytoskeleton keeps the plasma membrane under pressure, the well known surface tension. When cells, bathed in a physiological solution containing 1–2 mM concentration of Ca^{2+} are wounded at their surface, a major and quick influx of the cation, and thus a contraction of the membrane-associated cytoskeleton, take place. Under these conditions the edges of the wound separate from each other and therefore their spontaneous fusion becomes impossible (Fig. 1A,B). The only way the pressure can be balanced is by an increase of the surface membrane area, and this can be obtained only by exocytic fusion of internal membranes, with ensuing decrease of membrane tension. When this happens the edges of the wound become closer and their spontaneous fusion can thus take place (Fig. 1C).

The geometry of the exocytic fusion has been a matter of intense discussion. Initially it was proposed that organelles fuse to each other within the cytoplasm, giving rise to large "membrane patches" able to be transferred to the surface and to seal the wound. Although apparently very convenient, this mechanism soon appeared incompatible with basic properties of membranes. The mutual fusion of organelles, in fact, is not expected to yield patches but rather vacuoles whose insertion in the plasma membrane could not occur by some sort of simple surgical operation, as in the above hypothesis, but by exocytosis at well preserved areas of the plasma membrane. The latter process could be multiple, *i.e.* each participating organelle could undergo a Ca^{2+}-dependent exocytosis, with an ensuing enlargement of the plasma membrane corresponding to the sum of the exocytosed organelle surfaces [9-11] (Fig. 1C).

Summing up, according to the present understanding of the process, healing by the multiple exocytosis model (Fig. 1A-C) seems to occur most frequently in wounded cells. We cannot exclude, however, that large patches (or, more likely, large vacuoles) have also a role, in particular when the wounds are large and the affected cellular areas are rigidly organized as it is the case of echinoderm eggs and neuronal axons. Lesions of these structures appear to be followed by the local accumulation of multiple vesicular structures of variable size that could then fuse to participate in the healing process [12, 13].

Organelles involved

The general overview of the wound healing process presented so far (Fig. 1) requires the participation of cytoplasmic organelles competent for Ca^{2+}-dependent, regulated exocytosis. Since, as previously mentioned, healing appears to be ubiquitous, these organelles are expected to be expressed by all types of cells. The meaning of the regulated exocytic process is not always clear in the scientific community. Most often, regulated exocytosis is taken as regulated secretion, a process that however occurs only in specialized cells: neurons, endocrine and exocrine cells. Therefore, although it is possible that in the above cells the specific secretory granules and vesicles participate in repair, these organelles cannot account for the process occurring in non-secretory cells, where they are not expressed. Based on these considerations, during the last few years, the attention has been focused primarily on organelles of another type, the lysosomes, which are ubiquitous and can therefore be able to play a repairing role in all cells.

Regulated exocytosis of lysosomes has been widely discussed. On the one hand there is no doubt that various types of cells express lysosomes

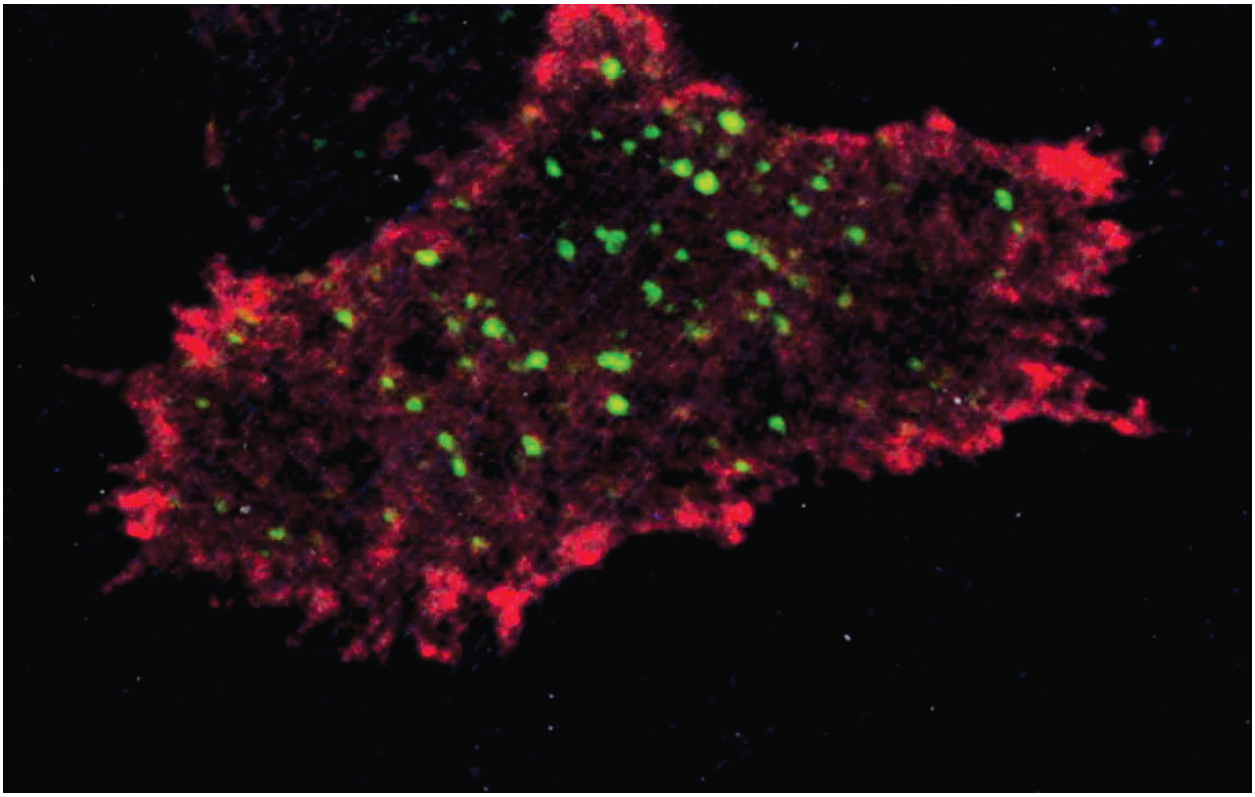

Fig. 2 Merged immunocytochemical image of enlargeosomes (red) and endosomes (green) in a defective PC12 cell [15, 17] investigated by confocal microscopy.
Before fixation and immunolabeling the cell was stimulated with the Ca^{2+} ionophore, ionomycin (5 μM, 2 min). The immunolabeling for the enlargeosome marker [17] appears therefore not only as red cytoplasmic puncta distributed especially in the proximity of the plasma membrane, as in resting cells, but also at the plasma membrane itself where it has been transferred as a consequence of exocytosis. Early endosomes, immunolabeled for their marker, EEA1, are distributed in deeper areas of the cytoplasm. No co-immunolabeling of the two antigens (which in the conditions employed would have appeared coloured in yellow) is visible. Lack of co-immunolabeling (and therefore of common nature) with enlargeosomes has been observed also after immunolabeling of other organelles: endoplasmic reticulum, Golgi complex trans-Golgi network, lysosomes, late endosomes [17]. Enlargement: 600X.

specifically competent for the regulated process. In a previous section we have already mentioned the yolk granules of sea urchin eggs to which we can now add secretory lysosomes of many types of hematopoietic cells: platelets, neutrophils, eosinophils, macrophages and so on. Most cell types, however, are devoid of classical secretory lysosomes and yet appear to release lysosomal enzymes in a Ca^{2+}-dependent fashion. From this observation the group of Norma Andrews has concluded that all lysosomes are exocytic and that their fusion accounts entirely for the cell surface enlargement necessary for healing to take place. Moreover, the lysosome-mediated healing process appears to have a key role in a well known disease, trypanosomiasis [5].

In the lysosome hypothesis, however, a number of questions remains open. In at least three types of non-secretory cells: CHO and 3T3 fibroblasts and a secretion-defective clone of the pheochromocytoma PC12 cell line, the surface enlargement steps reported by capacitance assay following photolysis of caged Ca^{2+} trapped in the cytosol were small, corresponding to vesicles (<0.1μm in diameter) [14, 15], whereas the lysosomal diameter is on the average of 0.4-0.5μm. This result suggests that most exocytic organelles involved in wound healing are small vesicles. Moreover, recent results by Andrews in collaboration with the group of Simon have shown that Ca^{2+}-dependent exocytosis is a property not of all lysosomes, but only of those

already docked to the plasma membrane, which in various types of cells account for a largely variable fraction of those organelles (from 5 to 25%) [6]. Interestingly, exocytosis of lysosomes is not rapid but takes place, on the average, one minute or more after the exposure of the cells to the Ca^{2+} ionophore, ionomycin [6]. This delay is open to question. Healing, in fact, is known to take place within a few seconds from the lesion [16]. Finally, in a series of experiments carried out by my laboratory in collaboration with that of Tom Kirchhausen at Harvard, pretreatment of the cells with a new drug that blocks specifically exocytosis of lysosomes was found to leave wound healing unaffected (unpublished results).

As a whole, the results reported so far do not exclude the participation of lysosomes in the repair process. These results, however, strongly suggest the participation of at least another type of organelle, characterized by small size and rapid exocytic fusion in response to large increases of the cytosolic Ca^{2+} concentration.

In recent studies carried out with the defective PC12 cell clone mentioned above as well as with CHO, HeLa and other cell types (both lines and primary cultures), we have identified a new, small (60 nm in diameter) vesicular organelle, that we have called enlargeosome, that meets the requirements to be a participant in the wound healing process [17]. The discovery of the new organelle has been based, on the one hand, on the electrophysiological demonstration that regulated, Ca^{2+}-dependent exocytosis occurs not only in secretory but also in various, classically non-secretory cells [15]; on the other hand, on the identification of a molecular marker, a high molecular weight protein segregated in the vesicular lumen, which appears at the cell surface upon exocytosis [17]. Based on these studies we conclude that enlargeosomes are distinct from lysosomes, endosomes and all other types of cytoplasmic organelles (Fig. 2). Moreover, they appear to have unique functional and molecular properties. In particular, they are exocytosed quickly ($t_{1/2}$<1sec) and recycled from the cell surface very slowly [15]. Their fusion, therefore, induces rapid and persistent enlargement of the cell surface, as requested for wound healing. Moreover, their exocytosis is not affected by the drug blocking the exocytosis of lysosomes (unpublished results). At the moment we cannot exclude that other organelles, so far unidentified, participate in the wound healing process. Rather, the latter might involve any organelle competent for regulated exocytosis expressed by the wounded cell. The efficiency of the repair response could therefore depend on the nature and properties of these organelles as well as on their level of expression. What is clear is that organelles competent for regulated exocytosis are more numerous than believed until recently and that the contribution to wound healing of at least some of them (enlargeosomes; vesicles of skeletal muscle [7] and possibly others) appears of fundamental importance.

Conclusion

The study of wound healing has been important to clarify not only the process *per se* but also a number of aspects of cell biology and pathology that had remained undefined or completely unknown. On the one hand, the interest about new exocytic organelles of widespread distribution has already introduced new actors in the field, while others might appear soon; on the other hand, the identification of the process as necessary for cell survival has lead to the identification of a new target for possible development of drugs and therapeutics. At the cell biology level knowledge about wound healing is growing at fast rate, thus we expect a solid "state of the art" to be reached soon. In terms of pathology, in contrast, the information is still limited. Developments that appear most likely destined to occur soon are in genetic diseases [7] and aging [3]. At the moment mysteries are still numerous, waiting for new ideas and experimental approaches to be appropriately investigated and finally clarified.

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