Journal of Cell and Tissue Research Vol. 11(1) 2703-2708 (2011)
ISSN: 0974- 0910 *(Available online at www.tcrjournals.com)*

Review Article

DERMAL EXPOSURE OF NANOPARTICLES: AN UNDERSTANDING

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Received: March 2, 2011; Accepted: March 27, 2011

Abstract: Special surface properties of nano range particles have great interest now a day's. In this rapidly growing field, many nano size materials are produced for diverse applications starting from cosmetics to sensors. Possible occupational and accidental adverse health effects of these materials are so far scarcely investigated. Although dermal toxicity has been analyzed many times, this review emphasizes the local and systemic toxicity caused after dermal absorption of nanoparticles. Mechanism of absorption of nanoparticles and its co-relation with size have been discussed in this review. On the basis of existing literature, the potentially most relevant cellular target sites of nanoparticles, starting with nanoparticles uptake across the cell membrane, mechanisms of generation of reactive oxygen species and the activation of redox-sensitive signalling cascades are described. Finally, the precautions and safety measures require at the time of nanoparticle dermal usage have been discussed.

Key words: Nanoparticles, Dermal exposure

INTRODUCTION

Nanotechnology is one of the fastest developing areas of scientific research. Due to advanced development in the field of nanotechnology various nanomaterials are coming in contact with human in different applications. Nanoparticles can be divided into two large groups: not intentionally produced and engineered nanoparticles [1,2]. These are nanotubes, nanowires, nanoshells, niosomes, nano-emulsions, nanocapsules, nanosomes, liposomes, nanoparticles, quantum dots, dendrimers, fullerene, fluorescent dextran beads, soil particles (0.4-0.5μm) and biopolymers [3,4]. Presently, they are in use for many commercially available products like cosmetics and sunscreens, pharmaceuticals, stain resistant clothing, sports equipment, automobile catalytic converters, dental bonding, cleanings products, dressings for specific wound care strategies, but many are the fields of possible future applications of nanotechnologies as drug delivery systems, nano-medicine, environmental remediation, and cell imaging. Among these, nanoparticles could play an important role in cell labeling /targeting, skin wound healing therapies, nano-medicine, cosmetics and sunscreens etc. where skin is the target organ [5]. Such materials typically possess nanostructure dependent properties (e.g., chemical, mechanical, electrical, optical, magnetic, biological), which make them desirable for commercial or medical applications. Reports suggest that these properties are due to nanostructure-dependent biological activity that differs from bulk properties of the constituent chemicals and compounds as they have big surface-to-volume ratios, but such properties may lead to adverse effects on human health and environment too [1,2]. Royal Society and the Royal Academy of Engineering (2004)reported that nanop-articles should be treated as new chemicals from a risk-point of view because they can overcome with the body's normal protective barrier that is skin [2,6-8].

DERMAL ABSORPTION OF NANOPARTICLE

Major possible route of entry of nanoparticles is the absorption through skin [9], but other modes of entry can be inhalation, ingestion, voluntary injection, absorption, or implantation for drug delivery systems [10-]. Skin is often considered less permeable and the risk associated to this route is very low [14] but literature survey suggests that it is an important route of entry for nanoparticles both in occupational and accidental contact and it should be considered as risk evaluation [2,15-17]. Four pathways of penetration across the skin have been identified depending on physicochemical properties of the compound: intercellular, trans-cellular, and transappendageal like through hair follicles and sweat glands [2]. Various factors that influence the dermal absorption of nanoparticles can be divided into three groups: (i) location and skin conditions at the application site, (ii) physicochemical properties of the penetrating molecule, and (iii) physicochemical properties of the vehicle dispersing the penetrating molecule [27].

Location and Skin Conditions at the Application Site- The nature of the site of application of nanoparticle plays important role in absorption. It includes: (i) skin integrity and regional variation (ii) dimensions of orifices, aqueous pores, and lipidic fluid paths (iii) density of appendages set the basic conditions affecting the absorption of any agent. Skin integrity (i.e. thickness, presence of pores and follicles etc.) differs according to the site of the body [27]. Working with hairless animals did not show any penetration into the skin clearly indicating that the hair follicles might be an important pathway for skin invasion of particulate materials [18]. Skin or stratum corneum integrity may also be compromised by dermatological (e.g., atopic dermatitis,psoriasis, ichthyosis) and other pathological conditions (e.g., inflammation, burn, infections), damage and trauma, extensive use of detergents, and/or prolonged exposure to air conditioned, non-humidified environments [19-21]. The effects of flexing movement on uptake of nanoparticles on normal skin shows that mechanical flexion facilitated the penetration of micrometer-sized particles that were observed in deeper dermal layers. Also, other factors like lipophilic-hydrophilic gradient, pH gradient and isoelectric point have their vital role which influences dermal absorption of nanoparticles [5,22,23].

Physicochemical Characteristics of the Penetrating Molecule: Other important properties are of the nanoparticle itself which also effect the penetration of nanoparticle through the dermal barrier. Most important is the size, in healthy individuals nano-particles of size 40 nm in diameter and smaller have been successful in penetrating the skin stratum corneum through the lipidic intercellular route or aqueous pores, while larger nanoparticles i.e. larger than 40 nm do not penetrate the skin stratum corneum. Most particles that do penetrate will diffuse through skin cells, but some will travel down hair follicles (trans-follicular route) and reach the dermis layer [24-27]. The shape of the nanoparticle affects the permeability showing that spherical particles have a better ability to penetrate the skin than ellipsoidal particles because spheres are symmetric in all three special dimensions. One study compared the two shapes and recorded data that showed spherical particles located deep in the epidermis and dermis whereas ellipsoidal particles were mainly found in the stratum corneum and epidermal layers [26]**.** Other factors are as fallow:

(a) pKa of the penetrating molecule and pH of vehicle should be chosen accurately, since only the unionized fraction of a penetrating molecule will be transported to viable epidermis [27,28];
(b) Solubility and dissolved amount of the penetrating molecule in its vehicle [27,28];
(c) The partition coefficient of the penetrating molecule should be adequate as lipophilic molecule will easily partition in stratum corneum but will leave it with difficulty, whereas a hydrophilic molecule will suffer poor penetration [27-29];
(d) The molecular weight of a penetrating agent should be less than 600 Da to significantly permeate skin [27,30];
(e) The potential for binding and metabolism [31,32];
(f) The diffusion coefficient (D) of the penetrating molecule in its vehicle and in the skin [27].

Physicochemical Properties of the Vehicle Dispersing a Penetrating Molecule: There is a possibility of potential synergisms/interactions between dispersing vehicle, dispersed nanometric agent, and skin components . Therefore, the physicochemical properties of the dissolving or dispersing vehicle are of great importance [27]**.** The viscous formulations reduce the diffusion coefficient of the molecule in the vehicle, resulting in retardation of skin partitioning, and hence absorption. In addition,

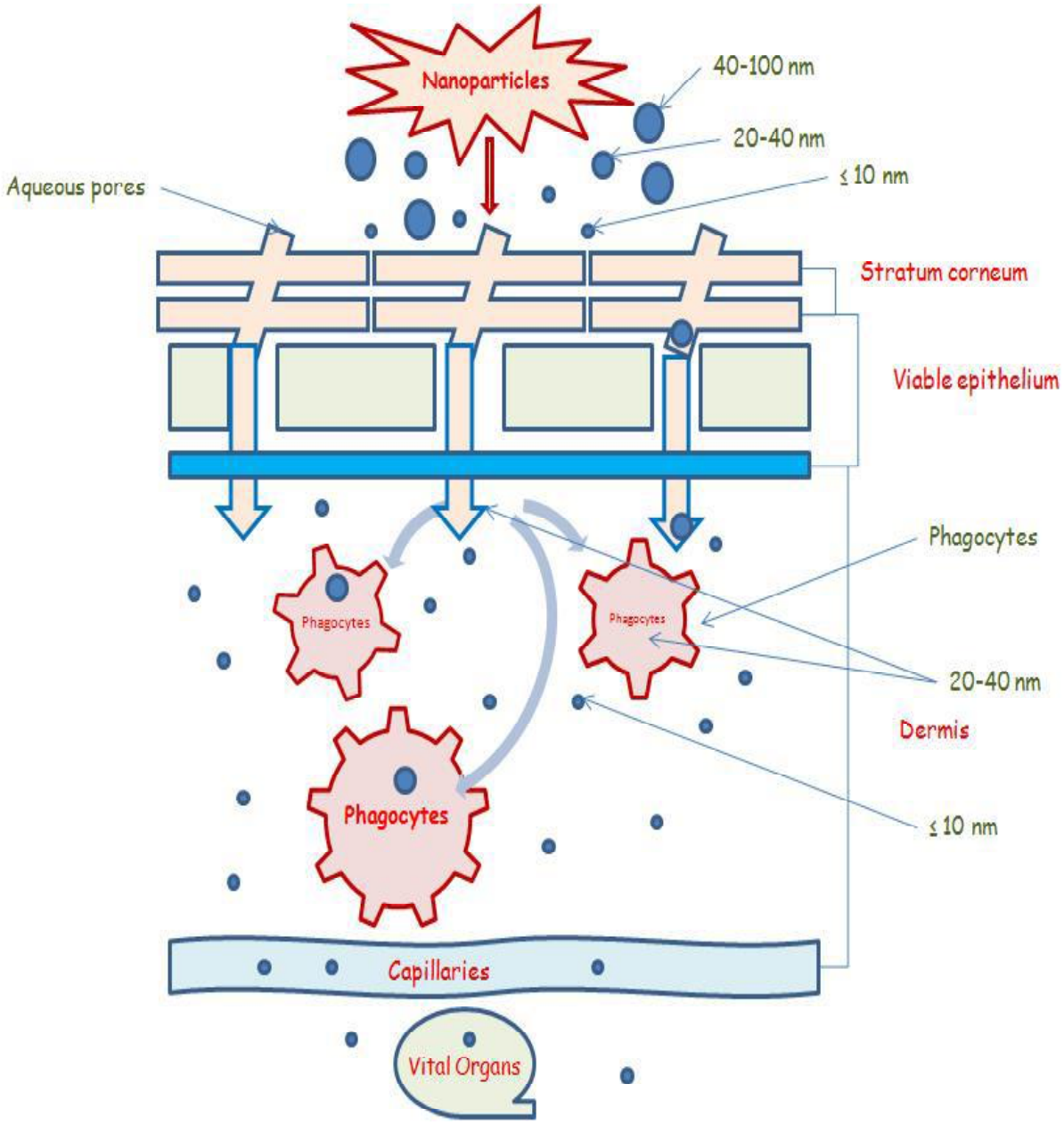

Fig. 1: Schematic presentation of dermal absorption of nanoparticle. Less than 10 nm nanoparticles can cross stratum corneum through diffusion and reached to dermis by intercellular or transcellular route while particle more than 20 nm can only be absorbed by transappendageal routes like hair follicle or aqueous pores. In d" 10 nm diameter nanoparticles, endocytosis hardly occurs, because the adhesion energy is too low to compensate for the bending energy. These nanoparticles do not engulfed by phagocytes and reached to blood circulation. Smaller nanoparticles (d" 10 nm) can easily cross cell membrane and interact with subcellular compartments

extremely lipophilic formulations compete with stratum corneum lipophilicity, hampering the SC partitioning of the penetrating agent but occlusive formulations may favor a moderate increase in absorption [27,33]. The presence of molecules such as solvents, surfactants, enhancers, and others may alter or damage SC by different processes thus causing a potential increase in the absorption of all or selected ingredients of the applied formulation [27,34,35].

Insertion of foreign agents in the stratum corneum (as well as their further progression toward the viable epidermis) is limited by stratum corneum nano-porosity and gradients. Accordingly, most important physicochemical parameters of penetrating agents appear to be dimensions, partition coefficient, and superficial properties. When penetrating agents are molecules, this reasoning leads scientists to indicate small (<600 Da) lipophilic and uncharged compounds as the best candidates for a successful percutaneous absorption [36-40]. The analysis of stratum corneum structure and nanoporosity firstly suggests that, in healthy individuals, these agents have to be smaller than 5–7 nm to have chances to diffuse— intact— throughout the fluid portion of the lipidic bilayers [39,41] or smaller than 40 nm to potentially use the aqueous pores [42-44] (Fig.1).

In addition, composition and physicochemical properties of tested nanometric agents as a whole, or of their components may further improve or limit the ingress and diffusion into/through the skin. These same properties may also be responsible for the maintenance of nanoparticle/nanomaterial integrity, when these agents enter in contact with skin components. In fact, it is reasonable to estimate that lipidic particles may interact to different extents with skin lipids, with which they may also fuse. It seems that trans-follicular penetration may be used by those agents whose dimensions are below follicular openings (i.e., 10–210 μm) and able to disperse themselves into sweat or sebum. Nonetheless, hair thickness should be subtracted from orifice diameter to evaluate the actual area (and volume) of the infundibulum that could be used for penetration or excretion. A homogeneous dispersion (without nanoparticle/ nanomaterial aggregation) seems to be a prerequisite for trans-follicular diffusion to occur. However, no stability or dispersibility data in sweat or sebum are reported in published articles.

DERMAL TOXICITY OF NANOPARTICLES

Dermal exposure of lesser size (< 10 nm) of nanoparticles is more disastrous than greater ones (> 30nm). It appears that lesser size nanoparticles can penetrate more easily than larger one. Reports suggest that nano-particle less than 10 nm showed prolonged erythema, oedema and eschar formation. Hyperkeratosis and papillomatosis in irregular epidermis and fibrosis, hyperemia, erythema, intracellular edema and hyelinisation of collagen in dermis are some histological observations in rabbit after exposing with nanoparticles of smaller size (<10nm). Further, bigger size nano-particle do not enter in skin even through transappendageal routes.

Possible pathways of cellular uptake of nanoparticles includes phagocytosis, macropinocytosis, clathrin-mediated endocytosis, nonclathrin-, non-caveolae-mediated endocytosis, caveolae-mediated endocytosis or diffusion [45-48]. During phagocytosis particles become engulfed via specific membrane receptors (e.g., scavenger receptors), leading to the formation of an early phagosome. Subsequent particle processing includes phagosome maturation which is described to be dependent on the involved receptor and may include the formation of a late phagosome and a lysosome. During particle ingestion via pinocytosis, a macropinosome is formed which also passes various maturation steps resulting into the formation of a lysosome. Clathrin-mediated endocytosis is performed due to specific membrane regions, referred to as clathrin coated pits. Following formation of a clathrin-coated vesicle and its uncoating with clathrin monomere recycling, particles are subsequently processed by early endosomes, multivesicular bodies and late endosomes. Endocytotic processes without involvement of clathrin or caveolin are referred to as non-clathrin noncaveolar- mediated endocytosis . Better known is the particle uptake via so called lipid rafts, leading to the formation of caveosomes with possible particle transcytosis, and transfer of the particles into the cytosol, endoplasmatic reticulum etc. Finally, particles may translocate into cells via diffusion, which in contrast to all aforementioned pathways, is a non-active process.

Physical interaction of nanoparticles with subcellular compartments are determinant of the type of reactive oxygen species generated. Interaction of nano-

particles with NADPH oxidase (NOX) complexes, mitochondria, or the endoplasmic reticulum, are reported [49]. Ca^{2+}ion release from calcium stores such as mitochondria and endoplasmic reticulum may be induced by local ROS-dependent loss of organelle membrane integrity and may result in activation of Ca^{2}/calmodulin-dependent enzymes, such as certain nitrogen monoxide (NO) synthase isoforms. There is a potential implication of ROS-mediated DNA damage by nanoparticles in mitochondria and the nucleus. Because of their ability to induce ROS and elicit oxidative stress within cells, nanoparticle may cause damage of the mitochondrial and nuclear genome respectively. Mitochondrial DNA damage may either result from direct actions of nanoparticle or by their interference with the electron transport chain, and has been implicated in the induction of apoptosis. Damage to the nuclear DNA can trigger various responses including cell cycle arrest, apoptosis or mutagenesis. Mitochondrial-derived ROS, or other signalling pathways may also impact on the integrity of the nuclear DNA [50].

CONCLUSION

Nanotechnologies are more a tool than a discipline, dedicated to manipulate the matter at nanometer-length scales, in order to produce new materials, structures, and devices. At this size range materials can have different mechanical and chemical properties than the same ones at larger size because of an increased relative surface area and quantum effects. It is becoming essential to assess nanomaterial skin absorption potential and its toxicity as, it is highlighted that the general public could come into contact with nanomaterial intentionally (e.g., drug delivery systems, various decontaminants, cosmetics etc). Even without entering into the details of exposure dose, length and repetition, it is clear that skin could be healthy, diseased, inflamed, hot, scratched/damaged, hydrated, moisturized and so on. These conditions offer different degrees of barrier impairment, and hence it cannot be excluded that nanomaterials could penetrate/ permeate skin better in one of these situations or can have various degree of effect. Therefore, special attention should be given to those formulations containing nanoparticles and intentionally applied to diseased skin to provide some therapeutic effects.

ACKNOWLEDGEMENT

Authors are thankful to Dr. S. J. S. Flora, Head of Division, for his support and suggestions.

REFERENCES

[1] Oberdo¨rster, G., Oberdo¨rsters, E. and Oberdo¨rster, J.: Environ Health Perspect, 113: 823–839 (2005a).
[2] Crosera, M., Bovenzi, M., Maina, G., Adami, G., Zanette, C., Florio, C. and Larese, F.F.: Int Arch Occup Environ Health, 82: 1043-1055 (2009).
[3] Website of the Royal Society and Royal Academy of Engineering on Nanotechnology and Nanoscience, Final Report atwww.nanotech.org.uk/ index.htm, August 2006;
[4] Nohynek, G.J., Dudour, E.K. and Roberts, M.S.: Skin Pharmacol & Physiol, 21: 136-149 (2008).
[5] Baroli, B., Ennas, M.G., Loffredo, F., Isola, M., Pinna, R. and Lopez-Quintela, A.: J Invest Dermatol, 127:1701–1712 (2007).
[6] Nasterlack, M., Zober, A. and Oberlinner, C., Int Arch Occup Environ Health, 81: 721–726 (2008).
[7] NIOSH, National Institute for Occupational Safety and Health (2007) Progress toward safe nanotechnology in the workplace—a report from the NIOSH Nanotechnology Research Center. DHHS (NIOSH). Pubblication No 2007-123, available online: http://www.cdc.gov/niosh
[8] Schulte, P.A., Geraci, C., Zumwalde, R., Hoover, M. and Kuempel, E.: J Occup Environ Hygiene, 5: 239–249 (2008).
[9] Chen, X. and Schluesener, H.J.: Toxicol Lett, 176: 1–12 (2008).
[10] Bianco, A., Kostarelos, K. and Prato, M.: Curr Opin Chem Biol, 9: 674–679 (2005).
[11] Guterres, S.S., Alves, M.P. and Pohlmann, A.R.: Drug Target Insights, 2: 147–157(2007).
[12] Klumpp, C., Kostarelos, K., Prato, M. and Bianco, A.: Biochim Biophys Acta, 1758: 404–412 (2006).
[13] Lademann, J., Richter, H., Teichmann, A., Otberg, N., Blume-Peytavi, U., Luengo, J., Weiß, B., Schaefer, U.F., Lehr, C.M., Wepf, R. and Sterry, W.: Eur J Pharm Biopharm, 66: 59–164 (2007).
[14] Rotoli, B.M., Bussolati, O., Bianchi, M.G., Barilli, A., Balasubramanian, C., Bellucci, S. and Bergamaschi, E.: Toxicol Lett, 178: 95–102 (2008).
[15] Fiserova-Bergerova, V., Pierce, J.T. and Droz, P.O.: Am J Ind Med, 17: 617–635 (1990).
[16] Nielsen, J.B. and Grandjean, P.: Am J Ind Med, 45: 275–280 (2004).

[17] Sartorelli, P., Ahlers, H.W., Alanko, K., Chen-Peng, C., Cherrie,J.W., Drexler, H., Kezic, S., Johanson, G., Larese Filon, F., Maina, G., Montomoli, L. and Nielsen, J.B.: Regul Toxicol Pharmacol, 49: 301–307 (2007).
[18] Shim, J., Kang, H.S., Park, W.S., Han, S.H., Kim, J. and Chang, I.S.: J Control Release, 97: 477- 484 (2004).
[19] Lavrijsen, A.P.M., Bouwstra, J.A., Gooris, G.S., Weerheim, A., Bodde´, H.E. and Ponec, M.: J Invest Dermatol, 105: 619–624 (1995).
[20] Suhonen, T.M., Bouwstra, J.A. and Urtti, A.: J Control Release, 59: 149–161 (1999).
[21] Farwanah, H., Raith, K., Neubert, R.H.H. and Wohlrab, J.: Arch Dermatol Res, 296: 514– 521 (2005).
[22] Wagner, H., Kostka, K.H., Lehr, C.M. and Schaefer, U.F.: Eur J Pharm Biopharm, 55: 57– 65 (2003).
[23] Elias, P.M.: J Invest Dermatol, 125: 183–200 (2005).
[24] Vogt, A., Combadiere, B., Hadam, S., Stieler, K., Lademann, J., Schaefer, H., Autran, B., Sterry, W. and Blume-Peytavi, U.: Journal of Investigative Dermatology, 126: 1316-1322 (2006).
[25] Sonavane, G., Tomoda, K., Sano, A., Ohshima, H., Terada, H. and Makino, K.: Biointerfaces, 65(1): 1-10 (2008).
[26] Ryman-Rasmussen, J.P., Riviere, J.E. and Monteiro-Riviere, N.A.: Toxicological Sciences, 91(1): 159-165 (2006).
[27] Baroli, B.: Journal of Pharmaceutical Sciences, 99: 21-50 (2010).
[28] Barry, B.W.: *Dermatological formulations.* Marcel Dekker, Inc., New York, pp 48–94 (1983).
[29] Moss, G.P., Dearden, J.C., Patel, H. and Cronin, M.T.: Toxicol In Vitro, 16:299–317 (2002).
[30] Barry, B.W.: Nat Biotechnol, 22: 165–167 (2004).
[31] Oesch, F., Fabian, E., Oesch-Bartlomowicz, B., Werner, C. and Landsiedel, R.: Drug Metab Rev, 39: 659–698 (2007).
[32] Merk, H.F., Baron, J.M., Neis, M.M., Obrigkeit, D.H., Karlberg, A.T .: Toxicol Appl Pharmacol, 224: 313–317 (2007).
[33] Kennish, L. and Reidenberg, B.: Dermatol Online J, 11: 7 (2005).
[34] Benson, H.A.: Curr Drug Deliv, 2: 23–33 (2005).
[35] Vavrova, K., Zbytovska, J. and Hrabalek, A.: Curr Med Chem 12: 2273–2291 (2005).
[36] Hadgraft, J. and Lane, M.E.: Int J Pharm, 305: 2– 12 (2005).
[37] Prausnitz, M.R., Mitragotri, S. and Langer, R.: Nat Rev Drug Discov, 3: 115–124 (2004).
[38] Hadgraft, J.: Int J Pharm, 224: 1–18 (2001).
[39] Johnson, M.E., Blankschtein, D. and Langer, R.: J Pharm Sci, 86: 1162–1172 (1997).
[40] Suhonen, T.M., Bouwstra, J.A. and Urtti, A.: J Control Release, 59: 149–161 (1999).
[41] Bouwstra, J.A. and Ponec, M.: Biochim Biophys Acta, 1758: 2080–2095 (2006).
[42] Cevc, G.: Adv Drug Deliv Rev, 56: 657–711 (2004).
[43] Tang, H., Mitragotri, S., Blankschtein, D. and Langer, R.: J. Pharm, Sci,, 90: 545–568 (2001).
[44] Potts, R.O. and Francoeur, M.L.: J Invest Dermatol, 96: 495–499 (1991).
[45] Mukherjee, S., Ghosh, R.N. and Maxfield, F.R.: Physiol Rev, 77: 759-803 (1997).
[46] Aderem, A., Underhill, D.M.: Annu Rev Immunol, 17: 593-623 (1999).
[47] Gruenberg, J.: Nat Rev Mol Cell Biol, 2: 721-730 (2001).
[48] Scott, C.C., Botelho, R.J. and Grinstein, S.: J Membrane Biol, 193: 137-152 (2003).
[49] Foley, S., Crowley, C., Smaihi, M., Bonfils, C., Erlanger, B.F., Seta, P. and Larroque, C.: Biochem Biophys Res Commun, 94: 116-119 (2002).
[50] Singh, K.K., Kulawiec, M., Still, I., Desouki, M.M., Geradts, J. and Matsui,S.: Gene, 354: 140-146 (2005).