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1 Anionic Quantum Dots reveal actin-microridges in zebrafish epidermis

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25 Abstract

26
27
28 Enhancement of the aqueous solubility and functionalization of CdTe-QDs (Quantum Dots) via surface
29 modifications have made them suitable to be used as specific probes for cell imaging. Applications for
30 targeting cell surfaces have been widely demonstrated *in vitro* but their use in animal models is not
31 trivial.

32 Here, we reported the interaction of mercaptosuccinic-coated (MSA) CdTe-QDs with the epidermis of
33 living and Carnoy-fixed zebrafish embryos.

34 QDs concentrate along adherent junctions and reveal the characteristic pattern of actin microridges at
35 the apical surface of the enveloping layer.

36 In our study, labeling with anionic QDs is attained within minutes at nanomolar concentrations in whole
37 mounted Carnoy-fixed zebrafish embryos, providing a faster approach compared with
38 immunodetection or standard Phalloidin staining of actin for visualization by fluorescence microscopy.

39
40 Keywords: actin-microridges, anionic quantum dots, zebrafish epidermis

1. Introduction

Nanotechnology has opened new possibilities to understand a variety of biological processes through the development of innovative nanosystems and techniques [1,2]. Since the '90s, Quantum Dots (QDs) have attracted increasing attention as an alternative nano-tool for life science, being applied in studies *in vitro* and *in vivo*, in biosensing, to monitor their interactions with cells or between biological molecules [1]. The development of hydrophilic QDs, coated with a chemically active surface for functionalization, has made these nanoparticles suitable as specific probes for imaging living biological systems as well as for other fluorescence-based bioassays [3]. QDs have already been conjugated with several classes of biomolecules, such as lectins and antibodies. These conjugates allowed, for example, to study cancer and microbial cell biology, taking advantage of the exceptional resistance to photobleaching suitable for long-term imaging [4,5]. Moreover, the blinking property of QDs has also been explored in super-resolution microscopy [6]. Studies in biological models *in vivo* and *in vitro* have been also conducted to assess the potential hazards of these materials [7][3].

The zebrafish is an ideal transparent model to apply new fluorescent probes for imaging. Despite the variety of functionalized QDs, their application as *in vivo* fluorescent probes in zebrafish remains scarce, and it has been limited to label vascular and neuronal systems [8,9]. This is in part due to physical restriction for targeting specific organs and tissues in adults or developed embryos.

Technically, the incorporation of nanoparticles or biomolecules in zebrafish is usually carried out by microinjection through the chorion at the one-cell stage [10], or in the heart ventricle after 24 hpf (hours post fertilization) [9]. At later stages, uptake of non-targeted QDs is most likely to occur via the gastrointestinal tract [11]. In any case, the final fate of the nanoparticles largely depends on their size, shape, and surface chemistry [12,13].

Zebrafish epidermis has been used as a tissue model for epithelial studies mainly due to its structural and functional homology to human skin [8,14,15]. In particular, zebrafish develops a bilayered epidermis within 24 hpf, constituted by the epidermal basal layer (EBL) and the enveloping layer (EVL). After three weeks, the bilayered epidermis becomes further stratified [16,17]. During epidermis morphogenesis, the apical zone of the EVL extends membrane projections known as microridges [18–20]. Recent microscopy studies support the idea that the patterned microridges organize the surface glycocalyx and protective mucus layer [17,21]. Besides, studies of microridge dynamics suggest that these patterned structures could act as a stable reserve of pre-polymerized F-actin readily available for cellular remodeling during epithelial wound healing processes [22].

In the present paper, we explored the interaction of exogenously added mercaptosuccinic-coated CdTe QDs with the embryonic bilayered epidermis of living and Carnoy-fixed zebrafish embryos.

2. Experimental

2.1 Quantum dots synthesis and optical characterization

CdTe QDs were synthesized in an aqueous colloidal dispersion stabilized with 3-mercaptopropionic acid (MSA). The nanocrystal average diameter and suspension concentration were estimated by UV-Vis spectroscopy following procedures described by Yu et al [23] and Dagtepe et al. [24]. Detailed calculations and characterization were carried out according to Tenório et al [5] with minor modifications. Briefly, QDs were prepared by the addition of Te^{2-} in a $\text{Cd}(\text{ClO}_4)_2$ solution at $\text{pH} > 10$ in the presence of MSA as the stabilizing agent in a 5:1:6 molar ratio of Cd:Te:MSA, respectively. All reagents used were acquired from Sigma-Aldrich. QDs were optically characterized by absorption (UV-Vis 1800 - Shimadzu) and emission (LS 55 - PerkinElmer, at $\lambda_{\text{exc}} = 488 \text{ nm}$) spectroscopies.

2.2 Zebrafish husbandry

A zebrafish (*Danio rerio*) strain of T/AB genetic background was used. Male and female adults were maintained at 28 °C, pH 7.0 - 8.0, on a 14 h light/10 h dark cycle. Adults and embryos were handled according to the ARRIVE guidelines and to the national guidelines from the Advisory Committee on Ethics of the *Facultad de Bioquímica y Ciencias Biológicas de la Universidad Nacional del Litoral*, Santa Fe, Argentina (Res. 229 and 388/2006).

After breeding, laid eggs were collected and maintained at 28 °C. Then, embryos and larvae were staged according to Kimmel et al. [25]. About 20 to 25 embryos were collected at 24, 31, and 48 hpf, then dechorionated and sedated with 0.01% buffered tricaine methanesulfonate (MS-222, Sigma-Aldrich). Embryos were then subjected to Carnoy fixation for immunodetection of E-cadherin and β -actin as well as to study the interaction with negatively charged CdTe QDs. Moreover, 20 live embryos of 10 and 24 - 28 hpf were dechorionated before incubation with QDs at 28 °C.

2.3 Immunodetection of E-cadherin and β -actin

Embryos were fixed *in toto* in Carnoy solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) overnight at 4 °C. Briefly, embryos were washed in phosphate-buffered saline (PBS, pH 7.4) and permeabilized in 1% Triton X-100/PBS for 1.5 h. Then, they were washed in PBS and incubated in normal goat serum (Vector Laboratories) for 45 min, followed by overnight incubation with primary antibody anti-E-cadherin, clone 36 mouse IgG2a, 610181, Transduction Laboratories) or anti- β -actin (monoclonal antibody, 8224, Abcam) at 4 °C. After three washes in PBS, embryos were incubated for 2 h with a secondary goat anti-mouse IgG-FITC antibody (Sigma-Aldrich, F8771) at 25 °C in darkness under orbital shaking. Finally, they were rinsed in PBS and mounted in 50% (v/v) glycerol-PBS for microscopy imaging. Embryos were also directly incubated with secondary antibody and normal goat serum as a negative control.

2.4 Incubation of live and fixed embryos with QDs

An aliquot of MSA-CdTe QDs suspension at pH 10 was adjusted to pH 8.0 with an aqueous solution of MSA (4.9% w/v). Then the suspension was rinsed using 10-kDa filters (GE Healthcare) and resuspended in PBS, or embryo medium, before incubation.

1
2
3 1 Living embryos were manually dechorionated and incubated in water pH 6.5 - 7.0 for 2 h at 28
4 2 °C with 0.7 μM QDs suspension. All treated embryos were rinsed and mounted in 1% low melting
5 3 agarose, and covered with 0.01% tricaine solution for image acquisition.

6 4 We treated two groups of fixed and permeabilized embryos (3-5 embryos per 1mL-polypropylene
7 5 tube). The first one was incubated only with QDs (50 μL , 0.7 μM final concentration), for 5, 10 and 30
8 6 min at 25 °C. The second group was incubated with QDs suspension under the same conditions, after
9 7 immunofluorescence detection (section 2.3). Then, embryos were thoroughly washed in PBS pH 7.4
10 8 and mounted in 50% (v/v) PBS-glycerol for at least 2 hours before image acquisition.

11 9 At the selected working concentration QDs labeling showed a high signal to noise ratio
12 10 during imaging, minimizing exposure times down to 100-200 msec for our microscope settings.

13 11 **2.5 Microscope settings and image acquisition**

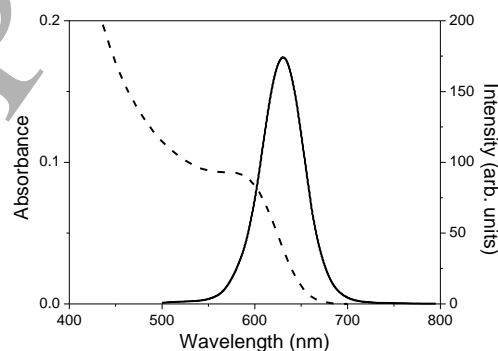
14 12 Images were acquired with an Olympus IX83 inverted wide-field sectioning microscope
15 13 equipped with a digital camera CMOS-ORCA-Flash 2.8 (Hamamatsu) and commanded by Olympus
16 14 CellSens software v. 1.13.

17 15 FITC and QDs signals were collected with ET - EGFP (FITC/Cy2, Chroma) and ET - DSRed
18 16 (TRITC/Cy3, Chroma) filter sets respectively. Lamp power was set at 6% and sampling in xy was
19 17 0.182 μm with z-step every 0.33 μm . At least 20 slices were collected to cover the epidermis bilayer
20 18 thickness (~6.6 μm). Epidermis analysis from z-stacks was performed using FIJI v. 3.0.

21 19 **3. Results and discussion**

22 20 **3.1 QDs optical characterization**

23 21 Red-emitting QDs were selected for simultaneous detection with green-emitting FITC-conjugated
24 22 antibodies. The emission wavelength of QDs was tuned by controlling the size of the growing crystals
25 23 during synthesis. Fluorescence spectrum of MSA-CdTe QDs aqueous suspension showed an intense
26 24 emission peak at 630 nm and a full width at a half maximum (FWHM) of about 53 nm (Fig. 1).
27 25 Moreover, based on the first absorption maximum peak at 582 nm, an average particle size of
28 26 approximately 3.4 nm was estimated. The concentration was estimated to be ca. 7 μM by absorption
29 27 measurements using the molar extinction coefficient for CdTe QDs.
30 28
31 29
32 30
33 31



32 **Figure 1.** Absorption (dashed line) and emission (full line) spectra of MSA-CdTe QDs, $\lambda_{\text{exc}} = 488 \text{ nm}$.

3.2 QDs interaction with live and fixed zebrafish embryos

The interaction of QDs with cell surfaces in biological systems has been discussed in length. Several factors influence this interaction, such as; size, shape and/or surface functional group and the biological system under investigation [26,27].

We sought to study the interaction of anionic red-emitting MSA-CdTeQDs with the epidermis of living zebrafish embryos after exogenous addition at two developmental stages, first during gastrulation and epidermis morphogenesis from 10 hpf and after 24 hpf, when the EVL covers the whole embryo and a bilayered epidermis is established. When QDs were incubated with embryos during gastrulation and allowed to develop up to 24 hpf, they were detected as scattered dots in the trunk (Fig. 2a) or head. In contrast, in embryos exposed to QDs for 2 h at 24 hpf, no labeling was attained. Only after generating a local physical injury of the epidermis, QDs immediately labeled the wounded area (Fig. 2b). QDs labeling localized to cell membranes and more faintly the cytoplasm of cells around the site of injury. This may be explained by the disruption of the protective mucus and of the negatively charged glycocalyx which may prevent the anionic MSA CdTe-QDs from binding to the intact epidermis *in vivo* [28].

In order to get access to the epidermal cells, embryos were fixed in Carnoy solution and permeabilized and then incubated with 0.7 μ M QDs solution for 5, 10 and 30 min at 25 °C (section 2.4). Carnoy solution has been described to preserve mucus[29] but its effect on the glycocalyx layer is not yet clear. Interestingly, we observed that within 5-10 minutes QDs labeled the cell membranes of the overlaying EVL, delineating cell-cell contacts and apical microridges (Fig. 2c-d). QDs labeling remained localized to microridges after extended incubation for 12 hours with 1.1 μ M QDs and did not penetrate beyond the EVL (Suppl. Information Fig. S1).

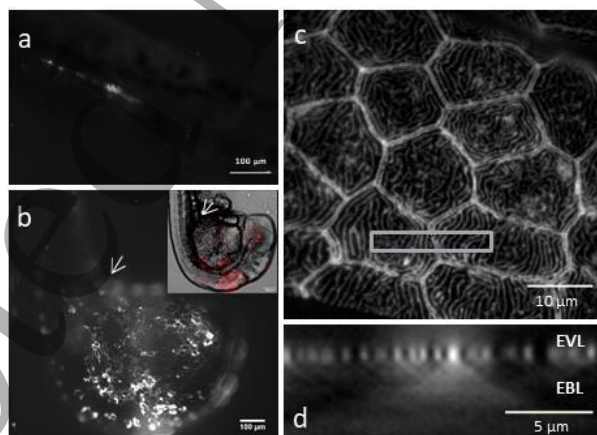


Figure 2. Exogenous addition of anionic MSA-CdTe QDs to live and fixed zebrafish embryos. **a)** Dispersed QDs were localized in the trunk of live embryos observed at 48 hpf (arrowhead) after 2 h incubation with QDs at 28 °C. The arrow indicates autofluorescence from yolk extension. This image is a maximum intensity projection of 66 slices. **b)** Live embryo (24 hpf), incubated with QDs for 2 h was labeled only upon physical injury at the yolk (arrow). **c)** Carnoy-fixed embryos at 48 hpf incubated with QDs, 30 min at 25 °C. **d)** xz projection of selected ROI in (c), covering the epidermis bilayer thickness, ~12 μ m, only cells of the EVL were labeled.

3.3 QDs label cell-cell contacts at the EVL but not the EBL

The embryonic epidermis is characterized by the expression of epithelial E-cadherin (E-cadh) outlining adherens junctions in both layers [30]. Based on the surface membrane labeling attained in fixed embryos with QDs, we analyzed whether the distribution of those nanoparticles correlates to that of E-cadh. Dual labeling was carried out by incubating embryos with QDs suspension after indirect IgG-FITC antibody immunodetection of E-cadh. Analysis of 3D-stacks across the epidermis showed that QDs fluorescence colocalized with E-cadh-FITC staining in the EVL layer but not in the underlying basal layer, EBL (Fig 3c-d and Movie 1 in Suppl.Information).

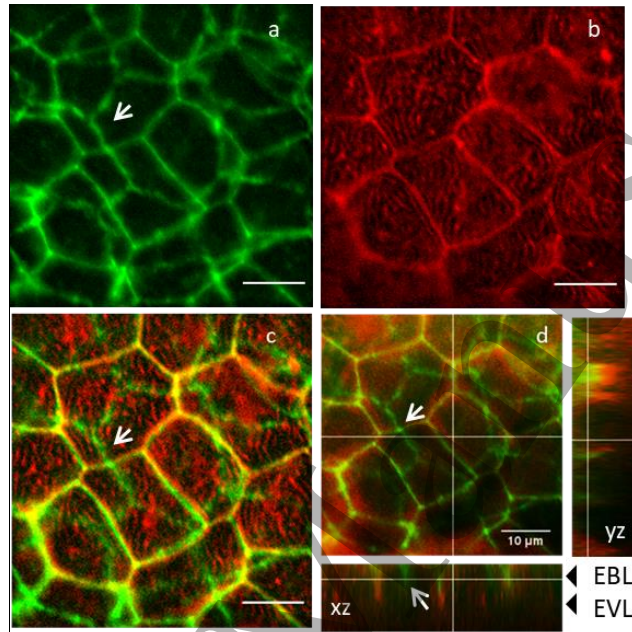


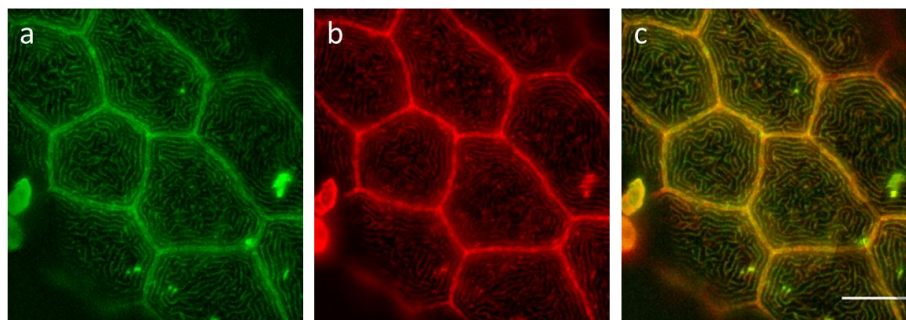
Figure 3. Dual labeling of membranes with QDs and E-cadh-IgG-FITC in embryos fixed at 24 hpf. **a)** E-cadh-IgG-FITC in the external EVL and underlying EBL (white arrow). **b)** Microridges labeled with red-emitting QDs on the EVL surface. **c)** QDs colocalize with E-cadh in EVL cells but are absent in the underlying EBL; **d)** Orthogonal views of 14 slices every 0.33 μm , covering $\sim 4.6 \mu\text{m}$ of epidermis thickness. Fixed embryos incubated with QDs, five minutes at 25°C, after immunostaining. Shown Images are contrast-enhanced maximum intensity z-projections. Green: FITC, Red: QDs em. 630 nm. Scale bar: 100 μm .

3.4 QDs colocalize with actin in cell-cell contacts and in apical microridges

Microridges are structurally constituted by filamentous actin and actin-binding proteins forming a highly dynamic network on the cell surface [20,22,31]. We then evaluated whether the observed QDs pattern correlated to that of F-actin. QDs colocalized with cortical actin in adherens junctions and with actin in apical microridges in fixed embryos of 48 hpf (Fig.4). These observations are consistent with the filamentous form of actin in these structures shown by standard Phalloidin staining [22].

Ai-Xue et al., showed that F-actin can interact electrostatically with negatively charged lipids using surface plasmon resonance and electrochemical impedance spectroscopy[32]. Thus, it is conceivable that Carnoy fixation may reduce carboxylates, sulfates and the sialic acid content of the

1 glyocalyx surface allowing MSA-CdTe QDs to interact with F-actin mediated by electrostatic forces.
 2 To our knowledge, this is the first description of microridges labeling with MSA-CdTe QDs as probes.



4
 5 **Figure 4.** Dual labeling of microridges in peridermal cells of fixed embryos at 48 hpf. **a)** green channel
 6 showing indirect IgG-FITC F-actin labeling; **b)** red channel showing labeling with QDs; **c)** overlay of
 7 green and red channels showing colocalization of QDs and F-actin at the membranes but partial
 8 overlap in microridges. Fixed embryos incubated with QDs, five minutes at 25°C after immunostaining.
 9 Shown are maximum intensity z-projections. Scale bar: 10 μ m.

11 Targeting the zebrafish epidermis with anionic QDs evidences that *in vivo*, the negatively charged
 12 glyocalyx and the protective mucus act as a first barrier, being impermeable to free nanoparticles.
 13 This was indeed evidenced after local injury of the epidermis and after fixation and permeabilization
 14 allowing for global membrane labeling of the whole embryo surface.

15 In zebrafish, actin microridges have been observed by gold standard Phalloidin staining [22] and by
 16 electron microscopy and live imaging [20] showing the overall arrangement of microridges as
 17 fingerprint-patterned structures on cell dorsal surfaces[14]. To our knowledge, this is the first
 18 description of labeling actin in microridges with non-targeted anionic MSA-CdTe QDs in zebrafish
 19 embryos. The QDs labeling approach is intended for *in toto* labeling of zebrafish embryos and larvae
 20 fixed in Carnoy. Additional high-resolution microscopy studies should provide further evidence of the
 21 true interaction with actin in other conformations and structures.

22 Microridges are not restricted to fish tissue and are indeed found in various mammalian epithelial
 23 tissues.-Recently, these structures have been getting considerable attention due to their dynamic
 24 organization during normal development and the alterations of the patterned structure in several
 25 epithelial-derived cancer cell types *in vitro* and during wound healing [33].

27 4. Conclusions

28 We have demonstrated here that, non-targeted 4 nm-sized anionic QDs proved suitable as a
 29 fluorescent probe for the identification of junctional actin in cell adhesions and the identification of F-
 30 actin supported microridges in the enveloping layer of zebrafish embryo epidermis.

31 QDs labeling was reproducible and specific for microridges structures, working from nano- to micro-
 32 molar concentrations and for incubation times varying from less than 10 min to 12 hours. In particular,
 33 red-emitting QDs were suitable for simultaneous detection with green fluorescent probes being as well
 34 as spectrally distinctive from endogenous fluorescence emission from the yolk.

1
2
3 1 The presented QDs labeling method is achieved within ten minutes at nanomolar concentrations being
4 2 compatible with classical immunodetection protocols and less time-consuming than standard
5 3 Phalloidin staining for F-actin.

6 4 Despite the ubiquitous presence of microridges in human epithelia, their role and relevance in the
7 5 normal organization of the surface glycan layer are still poorly known. In addition, alterations of
8 6 microridges patterns during the pathological transformation of epithelia have been long recognized,
9 7 but not exploited as diagnostic or therapeutic markers.

10 8 Only in the last decade, microridges were studied at the molecular level by high-resolution
11 9 fluorescence microscopy techniques. Our results indicate the potential of anionic QDs as fluorescent
12 10 probes capable of providing new information about actin-microridges which may help to elucidate
13 11 biological processes including normal epithelial morphogenesis and cancer progression.

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21 22 **Conflict of interests**

23 The authors have declared that no conflicting interests exist.

24 25 26 **References**

- 27 1. Wegner KD, Hildebrandt N. Quantum dots: bright and versatile in vitro and in vivo
28 fluorescence imaging biosensors. *Chem Soc Rev.* 2015;44: 4792–4834.
29 doi:10.1039/C4CS00532E
- 30 2. Pereira G, Monteiro C, Albuquerque G, Pereira M, Cabrera M, Cabral P, et al.
31 (Bio)conjugation Strategies Applied to Fluorescent Semiconductor Quantum Dots. *J Braz*
32 *Chem Soc.* 2019 [cited 13 Oct 2019]. doi:10.21577/0103-5053.20190163
- 33 3. Santos BS, Farias PMA, Fontes A. Semiconductor Quantum Dots for Biological
34 Applications. *Handbook of Self Assembled Semiconductor Nanostructures for Novel*
35 *Devices in Photonics and Electronics.* Elsevier; 2008. pp. 773–798. doi:10.1016/B978-
36 008046325-4.00026-8
- 37 4. Fontes A, Andrade C, Cabral Filho P, Tenorio D, Santos B, Beltrão E, et al. Evaluation of
38 glycophenotype in breast cancer by quantum dot-lectin histochemistry. *Int J*
39 *Nanomedicine.* 2013; 4623. doi:10.2147/IJN.S51065

- 1
2
3 1 5. Tenório DPLA, Andrade CG, Cabral Filho PE, Sabino CP, Kato IT, Carvalho LB, et al.
4 2 CdTe quantum dots conjugated to concanavalin A as potential fluorescent molecular
5 3 probes for saccharides detection in *Candida albicans*. *J Photochem Photobiol B*.
6 4 2015;142: 237–243. doi:10.1016/j.jphotobiol.2014.11.010
- 8
9 5 6. Fernández-Suárez M, Ting AY. Fluorescent probes for super-resolution imaging in living
10 6 cells. *Nat Rev Mol Cell Biol*. 2008;9: 929–943. doi:10.1038/nrm2531
- 12 7 7. Yong K-T, Law W-C, Hu R, Ye L, Liu L, Swihart MT, et al. Nanotoxicity assessment of
13 8 quantum dots: from cellular to primate studies. *Chem Soc Rev*. 2013;42: 1236–1250.
14 9 doi:10.1039/C2CS35392J
- 16 10 8. Li Q, Frank M, Thisse CI, Thisse BV, Uitto J. Zebrafish: A Model System to Study
17 11 Heritable Skin Diseases. *J Invest Dermatol*. 2011;131: 565–571. doi:10.1038/jid.2010.388
- 20 12 9. Son SW, Kim JH, Kim SH, Kim H, Chung A-Y, Choo JB, et al. Intravital imaging in
21 13 zebrafish using quantum dots. *Skin Res Technol*. 2009;15: 157–160. doi:10.1111/j.1600-
22 14 0846.2008.00341.x
- 24 15 10. Rosen JN, Sweeney MF, Mably JD. Microinjection of zebrafish embryos to analyze gene
25 16 function. *J Vis Exp JoVE*. 2009; 1115. doi:10.3791/1115
- 27 17 11. van Pomeran M, Brun NR, Peijnenburg WJGM, Vijver MG. Exploring uptake and
28 18 biodistribution of polystyrene (nano)particles in zebrafish embryos at different
29 19 developmental stages. *Aquat Toxicol*. 2017;190: 40–45.
30 20 doi:10.1016/j.aquatox.2017.06.017
- 33 21 12. Alivisatos AP, Gu W, Larabell C. Quantum Dots as Cellular Probes. *Annu Rev Biomed*
34 22 *Eng*. 2005;7: 55–76. doi:10.1146/annurev.bioeng.7.060804.100432
- 36 23 13. Williams Y. Surface Density of Charged Functional Groups on Quantum Dots
37 24 Determines Their Intracellular Compartmentalization and Biocompatibility. 2017; 12.
- 39 25 14. Rakers S, Gebert M, Uppalapati S, Meyer W, Maderson P, Sell AF, et al. ‘Fish matters’:
40 26 the relevance of fish skin biology to investigative dermatology. *Exp Dermatol*. 2010;19:
41 27 313–324. doi:10.1111/j.1600-0625.2009.01059.x
- 44 28 15. Eisenhoffer GT, Slattum G, Ruiz OE, Otsuna H, Bryan CD, Lopez J, et al. A toolbox to
45 29 study epidermal cell types in zebrafish. *J Cell Sci*. 2017;130: 269–277.
46 30 doi:10.1242/jcs.184341
- 48 31 16. Chang W-J, Hwang P-P. Development of zebrafish epidermis. *Birth Defects Res Part C*
49 32 *Embryo Today Rev*. 2011;93: 205–214. doi:10.1002/bdrc.20215
- 52 33 17. Le Guellec D, Morvan-Dubois G, Sire J-Y. Skin development in bony fish with particular
53 34 emphasis on collagen deposition in the dermis of the zebrafish (*Danio rerio*). *Int J Dev*
54 35 *Biol*. 2003;48: 217–231.
- 56 36 18. Zalik SE, Lewandowski E, Kam Z, Geiger B. Cell adhesion and the actin cytoskeleton of
57 37 the enveloping layer in the zebrafish embryo during epiboly. *Biochem Cell Biol*. 1999;77:
58 38 527–542.

- 1
2
3 1 19. Crawford BD, Henry CA, Clason TA, Becker AL, Hille MB. Activity and Distribution of
4 2 Paxillin, Focal Adhesion Kinase, and Cadherin Indicate Cooperative Roles during
5 3 Zebrafish Morphogenesis. *Mol Biol Cell*. 2003;14: 3065–3081. doi:10.1091/mbc.e02-08-
6 4 0537
- 8
9 5 20. Lam P, Mangos S, Green JM, Reiser J, Huttenlocher A. In Vivo Imaging and
10 6 Characterization of Actin Microridges. Weaver AM, editor. *PLOS ONE*. 2015;10:
11 7 e0115639. doi:10.1371/journal.pone.0115639
- 13
14 8 21. Rakers S, Niklasson L, Steinhagen D, Kruse C, Schaubert J, Sundell K, et al.
15 9 Antimicrobial Peptides (AMPs) from Fish Epidermis: Perspectives for Investigative
16 10 Dermatology. *J Invest Dermatol*. 2013;133: 1140–1149. doi:10.1038/jid.2012.503
- 17
18 11 22. Sharma A, Anderson KI, Müller DJ. Actin microridges characterized by laser scanning
19 12 confocal and atomic force microscopy. *FEBS Lett*. 2005;579: 2001–2008.
20 13 doi:10.1016/j.febslet.2005.02.049
- 22
23 14 23. Yu WW, Qu L, Guo W, Peng X. Experimental Determination of the Extinction
24 15 Coefficient of CdTe, CdSe, and CdS Nanocrystals. *Chem Mater*. 2003;15: 2854–2860.
25 16 doi:10.1021/cm034081k
- 26
27 17 24. Dagtepe P, Chikan V, Jasinski J, Leppert VJ. Quantized Growth of CdTe Quantum Dots;
28 18 Observation of Magic-Sized CdTe Quantum Dots. *J Phys Chem C*. 2007;111: 14977–
29 19 14983. doi:10.1021/jp072516b
- 31
32 20 25. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F. Stages of
33 21 embryonic development of the zebrafish. *Dev Dyn*. 1995;203: 253–310.
34 22 doi:10.1002/aja.1002030302
- 35
36 23 26. Filho P, Cabrera M, Cardoso A, Santana O, Geraldles C, Santos B, et al. Multimodal
37 24 highly fluorescent-magnetic nanoplatform to target transferrin receptors in cancer cells.
38 25 *Biochim Biophys Acta BBA - Gen Subj*. 2018. doi:10.1016/j.bbagen.2018.08.014
- 39
40 26 27. Jiang X-Y, Sarsons CD, Gomez-Garcia MJ, Cramb DT, Rinker KD, Childs SJ. Quantum
41 27 dot interactions and flow effects in angiogenic zebrafish (*Danio rerio*) vessels and human
42 28 endothelial cells. *Nanomedicine Nanotechnol Biol Med*. 2017;13: 999–1010.
43 29 doi:10.1016/j.nano.2016.12.008
- 45
46 30 28. Baskin JM, Dehnert KW, Laughlin ST, Amacher SL, Bertozzi CR. Visualizing
47 31 enveloping layer glycans during zebrafish early embryogenesis. *Proc Natl Acad Sci*.
48 32 2010;107: 10360–10365. doi:10.1073/pnas.0912081107
- 49
50 33 29. Johansson MEV, Hansson GC. Preservation of Mucus in Histological Sections,
51 34 Immunostaining of Mucins in Fixed Tissue, and Localization of Bacteria with FISH. In:
52 35 McGuckin MA, Thornton DJ, editors. *Mucins*. Totowa, NJ: Humana Press; 2012. pp.
53 36 229–235. doi:10.1007/978-1-61779-513-8_13
- 55
56 37 30. Sampedro MF, Izaguirre MF, Sigot V. E-cadherin expression pattern during zebrafish
57 38 embryonic epidermis development. *F1000Research*. 2019;7: 1489.
58 39 doi:10.12688/f1000research.15932.3

- 1
2
3 1 31. Pinto CS, Khandekar A, Rajasekaran B, Kiesel P, Pigo G, Sonawane M. Microridges
4 2 are apical projections formed of branched F-actin networks that organize the glycan layer.
5 3 bioRxiv. 2018 [cited 21 Feb 2019]. doi:10.1101/442871
6
7 4 32. Ai-Xue LI, Xiao-Qiang CUI, Fan YANG, Xiu-Rong YANG. Interaction Between F-Actin
8 5 and Negatively Charged Lipids Membrane. Chin J Anal Chem. 2009;37: 27–29.
9
10 6 33. Depasquale JA. Actin Microridges. Anat Rec. 2018;301: 2037–2050.
11 7 doi:10.1002/ar.23965
12
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