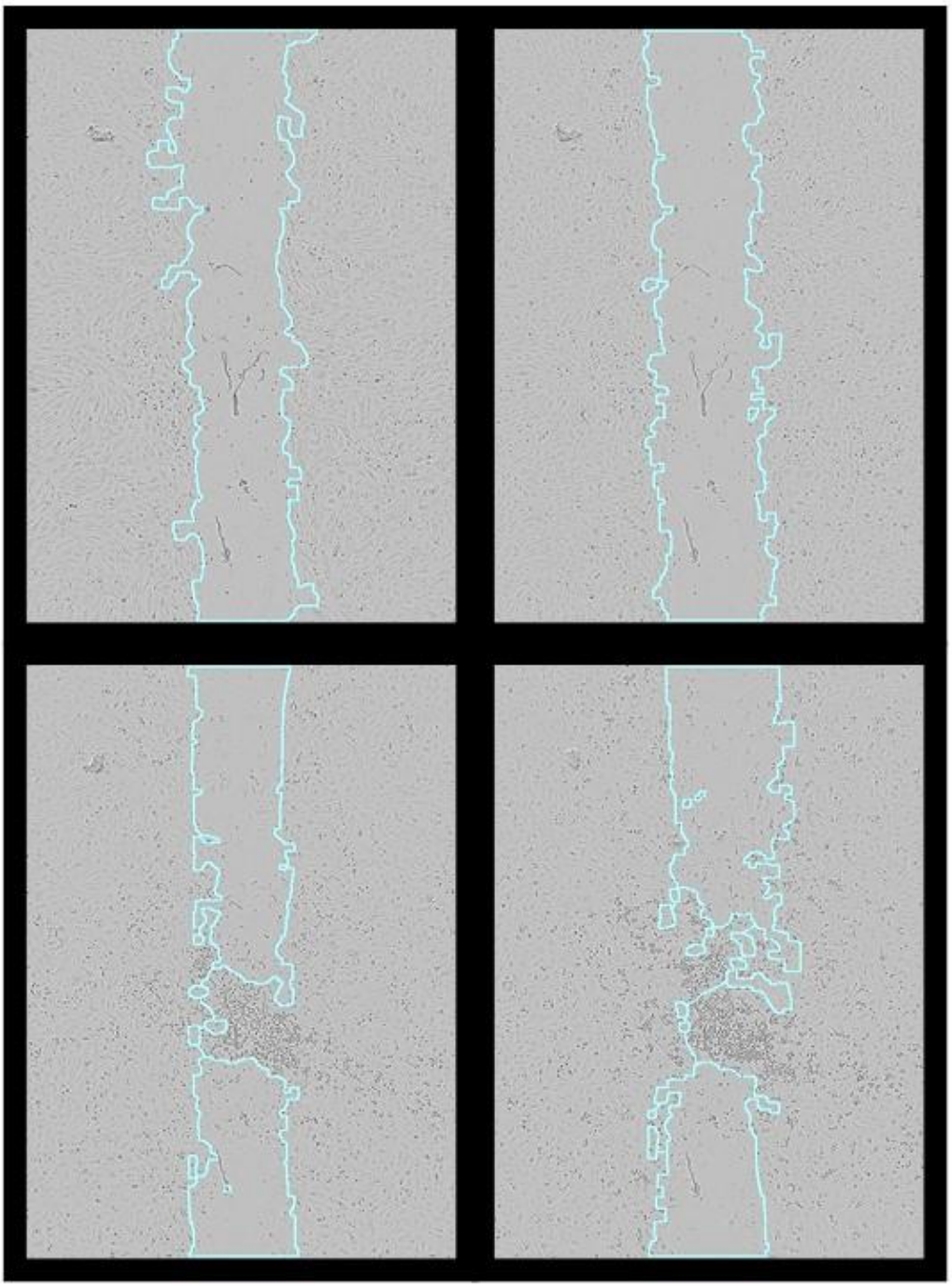
The applicability of nanodiamonds in collective cell migration studies



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Abstract

Collective cell migration is an important process in multiple fields within medical biology, namely embryonic development, cancer cell invasion and wound healing. Nanodiamonds could be useful in studying this process, as they have fluorescent properties, are extremely stable and can even detect magnetic fields. However, before they can be used in collective cell migration studies, the effects of the nanodiamonds themselves on cell migration should be determined.

In order to do this, Human Umbilical Vein Endothelial Cells (HUVEC) and triple negative breast cancer cells (MDA-MB-231) were used. Firstly, nanodiamond uptake was measured through confocal microscopy. Afterwards, the cell areas and aspect ratios were measured to determine if the nanodiamonds influenced the cells. Finally, the migration study was conducted by using gap closure assay.

In both the HUVECs and MDA-MB-231 cells, significant amounts of particles were detected compared to the controls. Nevertheless, there were no significant differences between the cell areas and cell aspect ratios of both cell types with nanodiamonds compared to the controls. In the cell migration experiment, significant differences were found 25 hours after insert removal, and only between some groups and the controls. In HUVECs, lower amounts of nanodiamonds increased migration speed, while high amounts (100 μg/ml) delayed migration. In the MDA-MB231 cells, higher amounts of nanodiamonds decreased migration speed more. However, significant differences may be detected earlier than 25 hours, since the previous statistically compared point was at 13 hours after insert removal.

Nevertheless, nanodiamonds do not seem to influence collective cell migration much, unless after a long period of time. More similar research needs to be done to determine the effects of nanodiamonds on different cell types, but a first step has been made, and hopefully the exciting characteristics of nanodiamonds will be useful in future collective cell migration studies.

Table of contents

Introduction

Collective cell migration is the coordinated movement of a group of cells influenced by their environment and their neighbouring cells[1]. This process is essential in multiple important themes within biomedical sciences, namely embryonic development, cancer cell invasion and wound healing[1, 2]. Although much has been revealed in the last decade, more remains to be explored. For instance, even if much is discovered about the chemical cues influencing collective cell migration, many mysteries remain concerning mechanical and physical cues[1]. To investigate collective cell migration, multiple approaches are available. Collective cell migration can be studied in 2D or 3D. For 2D approaches mainly gap closure analysis is used. In this approach the cells are seeded on a surface with a gap between the cell populations, and the diminishing of the gap over time is analysed using phase contrast microscopy[3, 4]. There are multiple methods available for 3D collective cell migration studies. To study it in vivo, one could use an animal model such as *Drosophila melanogaster*, as is done by Campbell et al., and for in vitro studies a transwell assay is commonly used[5-7]. However capable these methods are for studying the direction and speed of collective cell migration, they are unable to inform us about the exact conditions of the cells undergoing migration. For this end, markers are needed. One possible marker with special properties are nanodiamonds.

In biomedical sciences, nanodiamonds have shown two interesting properties. Firstly, they can be used as exceptionally stable fluorescent dyes[8, 9]. The nanodiamonds owe their capability to produce fluorescence to lattice defects, negatively charged nitrogen-vacancy centres for example[8-10]. Nanodiamonds can be used for long-term measurements, since they do not bleach, contrary to almost all other commonly used biolabels[9, 10]. Moreover, they are also biocompatible and inert[9, 10]. A plethora of studies showed the biocompatibility of nanodiamonds in a range of cell lines and even organs[11-16]. Another interesting property of nanodiamonds with negatively charged nitrogen-vacancy centres is their ability to record magnetic fields[8-10]. This can be measured as a drop in fluorescence when applying specific pulsing schemes.

Altogether, nanodiamonds seem promising in usage for collective cell migration studies. However, since nanodiamond imaging is a fairly new field, much is unknown. For instance, the exact location of the nanodiamonds in the cell is uncertain. Furthermore, nanodiamonds have to be tested on multiple cell types to determine if the diamonds themselves influence cell migration[7].

In order to illuminate the possibilities of using nanodiamonds in collective cell migration studies, this paper aims to determine if nanodiamonds influence collective cell migration in HUVEC and MDA-MB-231 cells. To answer this question, multiple aspects need to be examined. Firstly, the capability of the HUVEC and MDA-MB-231 cells to take up the nanodiamonds will be determined. Secondly, the influence of these nanodiamonds on the HUVEC and MDA-MB-231 cells will be measured. Lastly, the effect of the nanodiamonds on collective cell migration speed will be investigated.

Materials and methods

Cells and culture conditions

Triple negative breast cancer cells (MDA-MB-231) were maintained in DMEM complete composed of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4,5 g/L dextrose, 10% Foetal Bovine Serum (FBS) and 1% penicillin and streptomycin in a T75 flask at 37°C and 5% CO_2. Cells were sub-cultured twice per week at 90% of confluency. MDA-MB-231 cells were rinsed in phosphate-buffered saline (PBS) and detached from the bottom after 2 min treatment with 2 ml trypsin/EDTA at 37°C. Cells were resuspended in new T75 flasks containing DMEM complete.

Human Umbilical Vein Endothelial Cells (HUVEC) were cultured in EBM-2 medium supplemented with EGM-2 MV Single Quot Kit Supplements and Growth factors (Lonza) at 37°C with 5% CO_2/95% air conditions at the UMCG Endothelial Cell Facility.

Sample preparation

MDA-MB-231 cells at 90% of confluency were detached using trypsin/EDTA and resuspended in DMEM complete. At the same time, HUVECs in suspension in EBM-2 complete medium were acquired from the UMCG Endothelial Cell Facility. Both MDA-MB-231 cells and HUVECs were counted using a haemocytometer. Then 15.000 cells were transferred into each well of the 2-Well insert (*Ibidi*) placed on a 35 mm glass-bottom dish coated with 10 µg/ml human fibronectin completing a volume of 70 ul per well with DMEM or EBM-2 complete, respectively. Cells were incubated for 24 h in DMEM or EBM-2 complete, respectively, at 37°C and 5% CO_2, until they formed a confluent monolayer (30.000 cells per well). At this point, MDA-MB-231 cells and HUVECs were incubated with 70 nm fluorescent nanodiamonds (FNDs) previously resuspended in FBS (final concentration 10%) and then diluted in DMEM or EBM-2 serum-free. The FNDs were used in concentrations 1, 10 and/or 100 µg/ml. Next, cells were incubated with FNDs for 4h or 24h at 37°C and 5% CO_2, as described in Figure 1 and 2. Cells cultured in DMEM or EBM-2 complete, respectively, without nanodiamonds were used as a negative control.

After incubation with nanodiamonds, cells were rinsed twice with PBS and cultured in DMEM or EBM-2 serum-free (medium without FBS) for 16 h at 37°C and 5% CO_2. Then the insert was removed using tweezers, and the medium was replaced by DMEM or EBM-2 low serum (medium supplemented with 1% FBS).

Cell uptake study groups

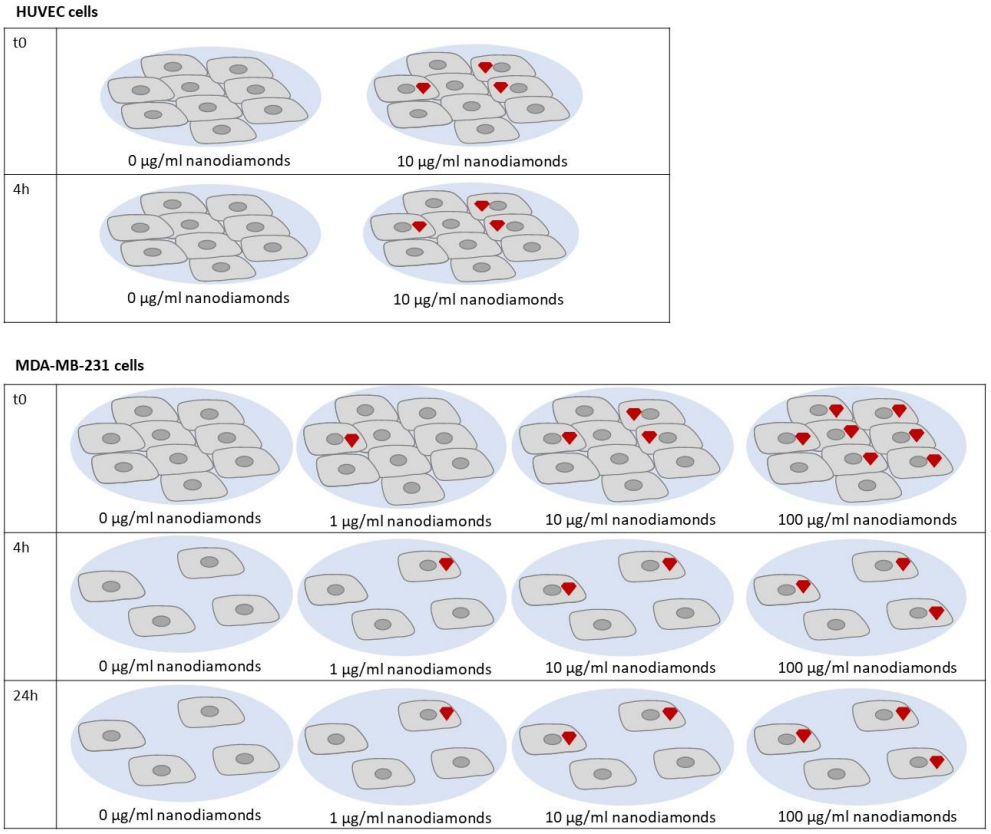

Figure 1: Groups used in the cell uptake study. For the HUVECs, two different concentrations were used per timepoint. For the MDA-MB-231 cells, four different concentrations were used per timepoint. 4h and 24 h means cells were incubated with the nanodiamonds for respectively four hours and twenty four hours. t0 means the cells have incubated with the nanodiamonds for four hours, then were rinsed and incubated for sixteen hours in medium without serum. The images of dense cells represent cells grown in monolayers, and the sparsely populated images represent cells grown as single cells.

Migration study groups

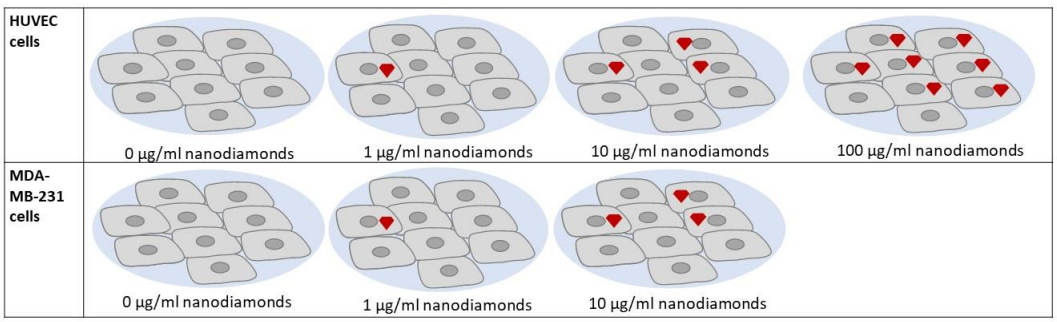

Figure 2: Groups used in the migration study. Cells were incubated for four hours with nanodiamonds. Four different nanodiamond concentrations were used in the HUVECs, and three in the MDA-MB-231 cells. Each group was followed for 24 hours.

Cell uptake study

To study the uptake of FNDs, cells at conditions described in Figure 1 were immunostained. MDA-MB-231 cells and HUVECs were fixed in 4% paraformaldehyde and permeabilized in 0,5% Triton X-100 in PBS. After 30 min blocking in 0,5% bovine serum albumin in PBS, cells were incubated with a mouse-anti-human vinculin primary antibody for 1 hour at room temperature. Secondary antibody goat-anti-mouse IgG H&L conjugated with FITC and DAPI were incubated for 30 min at room temperature. Confocal stack images were acquired in *Zeiss LSM780* using a 63xW objective. Images were acquired using lasers in the Excitation/Emission range of 358⁄461 nm for DAPI, 488/520 nm for FITC and 532/700 nm for FNDs voxel size of 200 x 190 x 190 nm.

Migration study

Brightfield imaging was performed 1 h after insert removal using an IncuCyte S3 Live-Cell Analysis System. Cells were maintained for 24 h at 37°C and 5% CO_2 while images were acquired each 30 min using 4x objective.

Image analysis

Fiji (National institutes of health, Bethesda, MD) was used to process all acquired images[17, 18]. To quantify nanodiamond particles inside the cells, confocal images were analysed using a methodology previously described[16]. Additionally, the confocal images were analysed to detect cell borders in order to determine the cell area and the aspect ratio of the cells. Brightfield image stacks were processed to detect gap closure[17]. The gap area of the initial timepoint was set to 100%, and the gaps of the remaining timepoints were calculated as percentages relative to the initial timepoint within a sample. Thereafter, differences between resulting percentages and initial timepoints were computed and the data from samples within a group were averaged. Resulting data were plotted and analysed using Graphpad Prism 7.00.

Statistical analysis

A two-way ANOVA was used in Graphpad Prism 7.00 to analyse both the cell uptake study and migration study data. For the cell uptake study, comparisons were made between different concentrations within timepoints, and between timepoints of similar concentrations within a cell type. Concerning the migration study, a few timepoints were selected to compare, namely t0, t2, t12, and t24. The different concentrations administered to a cell type were compared within a timepoint. Results were considered statistically significant if $p < 0,05$ (n.s. $p > 0,05$; *$p < 0,05$; **$p < 0,01$; ***$p < 0,001$; ****$p < 0,0001$).

Results

Cell uptake study

In the cell uptake study, the capability of HUVECs and MDA-MB-231 cells to take up nanodiamonds and the influence of the nanodiamonds on cell area and aspect ratio were determined. Firstly, in the HUVECs highly significant amounts of objects and particles were detected in the t0 10 µg/ml sample compared to the other samples (Fig. 3a, b). However, this did not lead to a significant difference between the area of the groups, only between the two control groups, finding smaller cells at time 4h than t0 (Fig. 3c). Furthermore, there was also no significant difference between the aspect ratio of the cells and their corresponding control groups, but there was between the different timepoints of equal nanodiamond concentrations, which means that cells at 4h are more elongated than cells at time t0 (Fig. 3d). For the MDA-MB-231 cells, significantly higher amounts of objects and particles were found in cells administered 100 µg/ml for four hours, 10 µg/ml for twenty four hours, and 100 µg/ml for twenty four hours (Fig. 4a, b). Nevertheless, no significant difference whatsoever has been detected in the areas and aspect ratios of the cells (Fig. 4c, d).

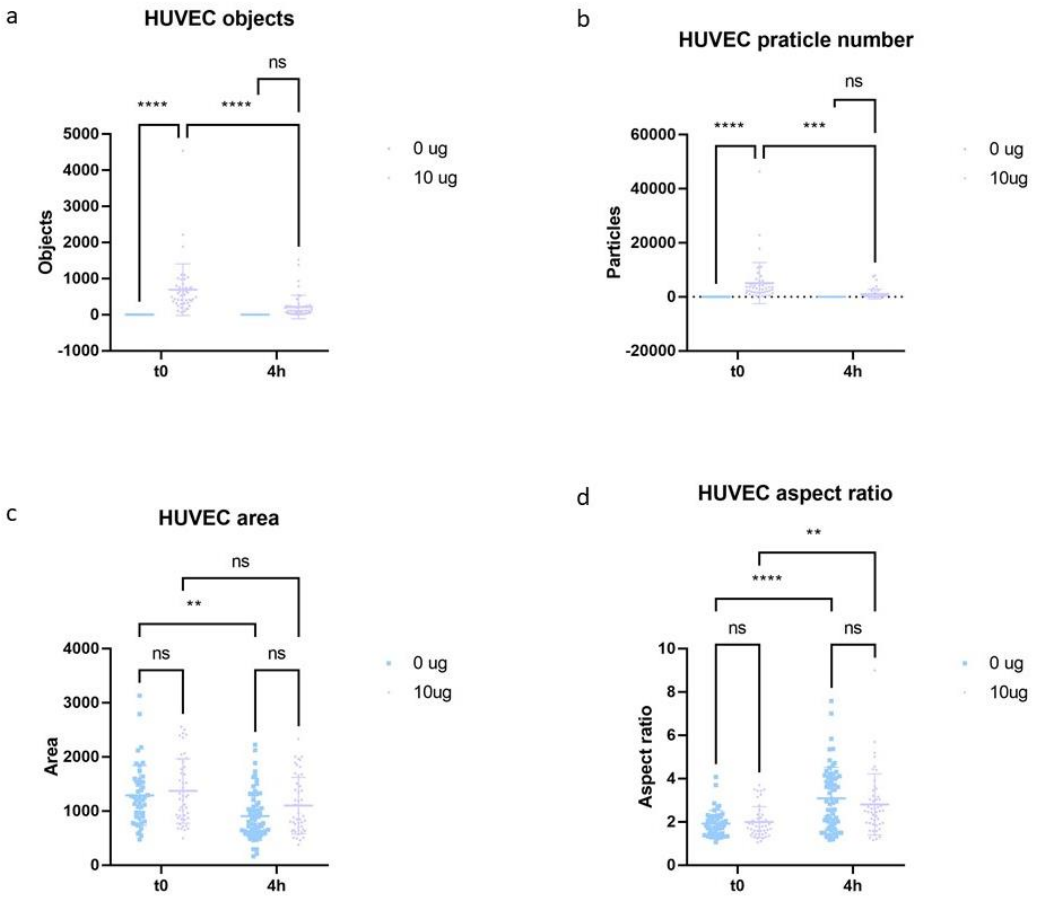

*Figure 3: Cell uptake study of the HUVECs. (a) shows the amount of objects, or nanodiamond clusters, observed inside the cells. (b) shows the amount of nanodiamonds inside the cells. (c) indicates the area the cells cover. (d) illustrates the aspect ratio of the cells. n.s. p > 0,05; *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.*

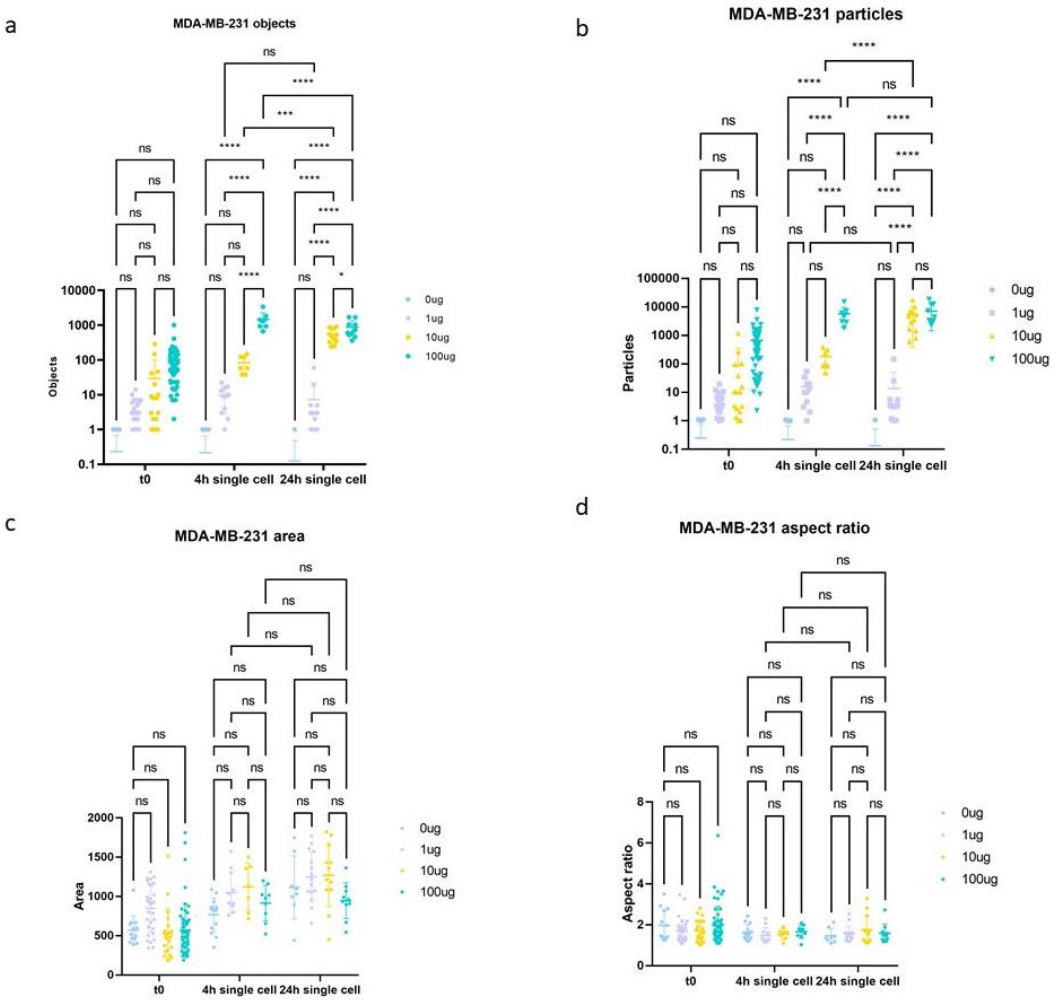

*Figure 4: Cell uptake study of the MDA-MB-231 cells. (a) shows the amount of objects, or nanodiamond clusters, observed inside the cells. (b) shows the amount of nanodiamonds inside the cells. (c) indicates the area the cells cover. (d) illustrates the aspect ratio of the cells. n.s. p > 0,05; *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.*

Migration study

Gap closure analysis was performed to determine the rate of gap closure of the HUVEC and MDA-MB-231 cells. In all groups, some form of gap closure was observed (Fig. 5). In HUVECs, lower doses of nanodiamonds (1 µg/ml, 10 µg/ml) increase the gap closure rate with respect to the control, while a high dose of 100 µg/ml decreases the gap closure rate (Fig. 6a). However, significant statistical difference is only found late in the experiment, at twenty five hours after insert removal, and just between the control and 1 µg/ml, 1 µg/ml and 10 µg/ml, and 10 µg/ml and 100 µg/ml (Fig. 6c). In the MDA-MB-231 cells, all groups closed the gap steadily, with the control group in the lead, followed by 1 µg/ml, and lastly 10 µg/ml (Fig. 6b). Here as well significant differences were only found at twenty five hours, and only between the control and 10 µg/ml (Fig. 6d). When all groups of the two cell types are compared, it is noticeable that the MDA-MB-231 samples are grouped around the control group of the HUVECs (Fig. 7).

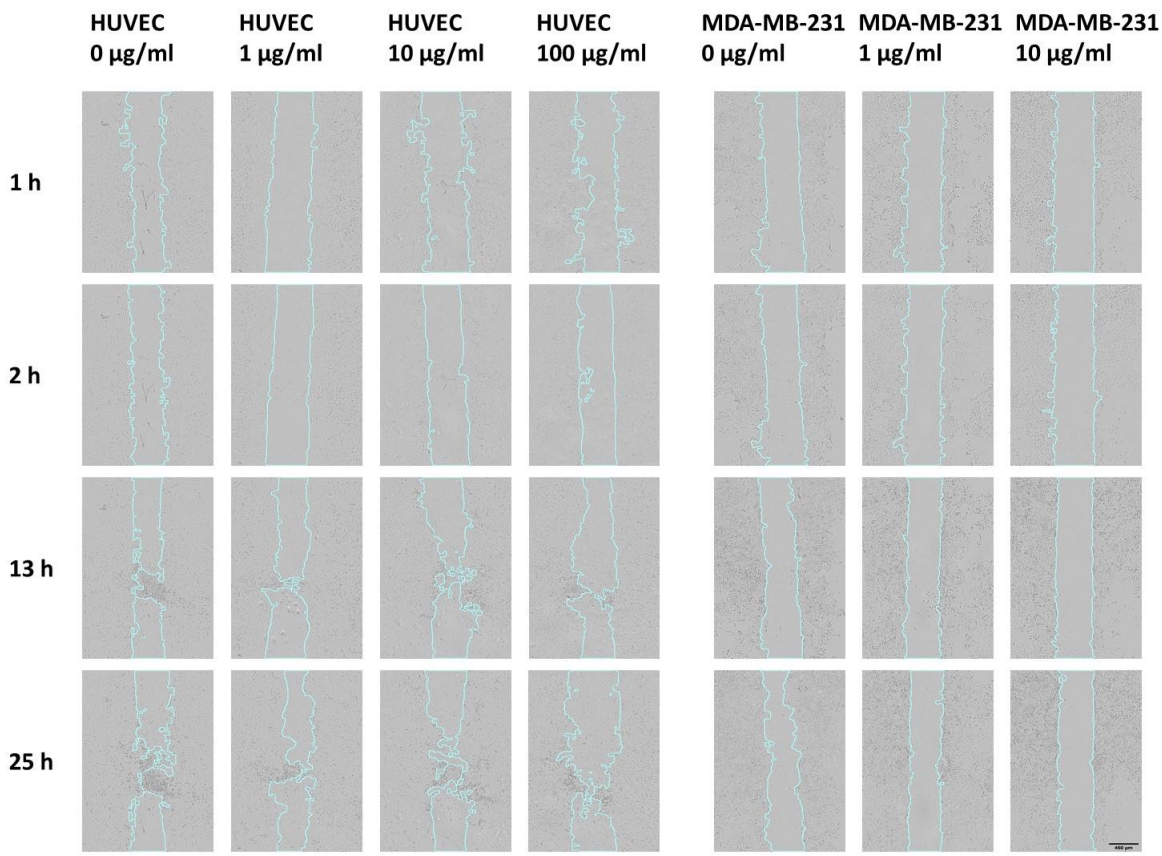

Figure 5: Images of the migration study. Left are the timepoints the pictures were taken, in hours after insert removal. Above are the cell types with concentrations of nanodiamond. Gaps are between the blue lines. In the lower right corner is a scalebar of 450 µm.

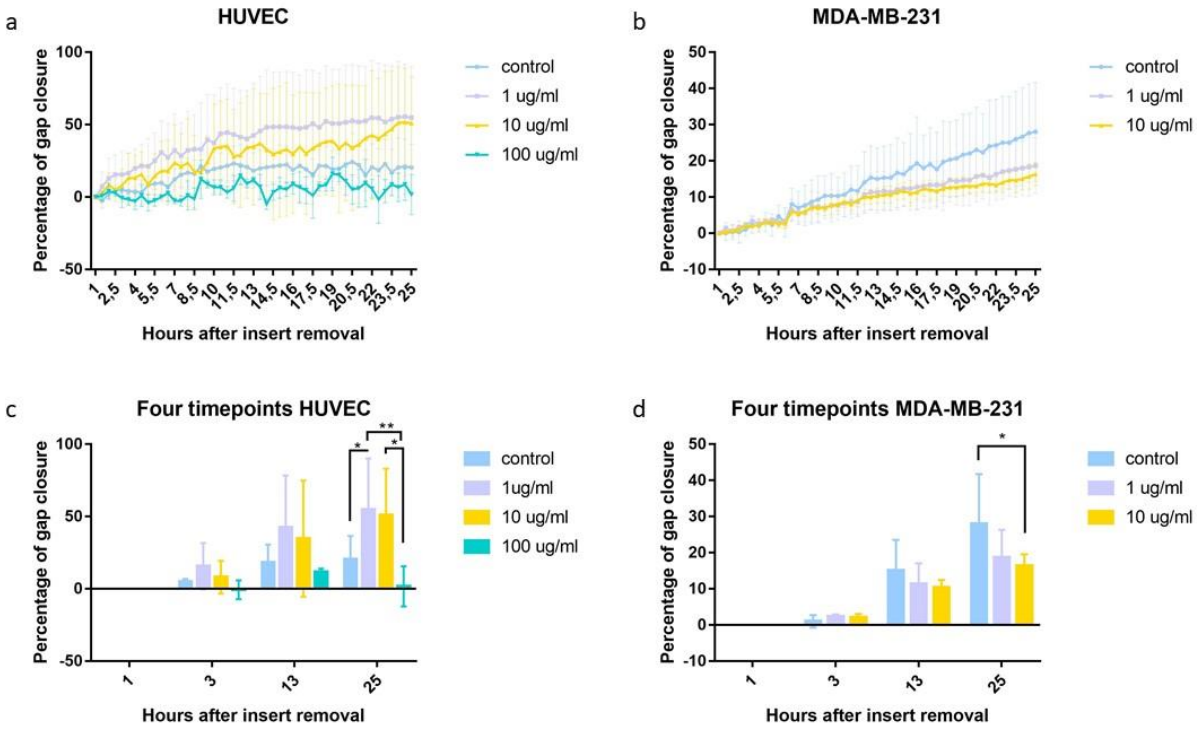

*Figure 6: Gap closure rate of HUVEC and MDA-MB-231 cells. The percentage of gap closure is the difference between a timepoints gap area and the gap area at t0, with t0s area being set to 100%. (a) illustrates all timepoints for the HUVECs. (b) shows all timepoints for the MDA-MB-231 cells. (c) exhibits four timepoints of the HUVECs, with significance calculations. d illustrates four timepoints of the MDA-MB-231 cells, with significance calculations. n.s. p > 0,05; *p < 0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001.*

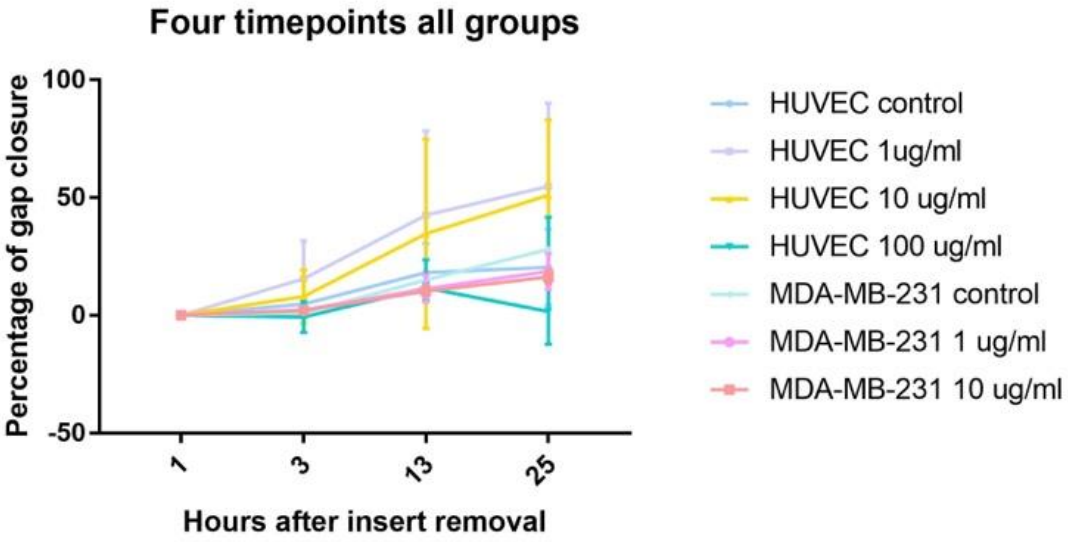

Figure 7: Gap closure rates for four timepoints of all groups of both HUVEC and MDA-MB-231 cells. Percentage of gap closure is the difference between a timepoints gap area and the gap area at t0, with t0s area being set to 100%.

Conclusions and discussion

Both cell types showed significant uptake of nanodiamonds (Fig. 3a, b; 4a, b). In the HUVECs, nanodiamond uptake was significant compared to the control group after 16 h serum deprivation, but not before. This could be because the program does not count particles bound to the cell membrane. After 16 h of serum deprivation those particles could have migrated inside the cytoplasm, and thus be counted. In the MDA-MB-231 cells, significant nanodiamond uptake in regard to the control groups was measured in three groups, namely 4 h incubation with 100 µg/ml, 24 h incubation with 10 µg/ml, and 24 h incubation with 100 µg/ml. The other groups also appeared to have increased nanodiamond levels compared to their controls, but these increases were not significant with the statistical tests used. Furthermore, the nanodiamonds did not significantly affect the areas and aspect ratios of the cells (Fig. 3c, d; 4c, d). For the HUVECs, there was only significant differences in area and aspect ratio between the timepoints, which then could be ascribed to the 16 h serum deprivation. For the MDA-MB-231 cells, there was no significant difference anywhere to be found. Finally, nanodiamonds influenced cell migration, but the significance was minimal and at later timepoints (Fig. 6). In the HUVECs, lower concentrations of nanodiamonds (1 µg/ml and 10 µg/ml) lead to an increase in cell migration related to the control, and 100 µg/ml to a decrease. Only 1 µg/ml showed a significant difference with the control at 25 h after insert removal, at $p < 0,05$. In MDA-MB-231 cells, both nanodiamond concentrations decreased cell migration compared to the control, with a stronger effect noted in 10 µg/ml than in 1 µg/ml. For these cells as well, significant difference was only measured at 25 h after insert removal between the control and 10 µg/ml at $p < 0,05$. However, the differences could also be significant at earlier timepoints (between 13 h and 25 h), as those measurements have not been compared. Furthermore, especially for MDA-MB-231 cells, the differences seem to increase over time. Thus, if measurements continued indefinitely, larger differences between the groups could possibly be found. Additionally, the effects of nanodiamonds on cell migration also differ per cell type (Fig. 7). The MDA-MB-231 groups stay close together compared to the HUVECs. This once again shows that experiments for one cell type are not representative for all.

It is no surprise that HUVECs can take up nanodiamonds, as it has been done before. The nanodiamonds used by Gerstenhaber et al. where even larger than those used in our experiments, namely ~800 nm compared to 70 nm[11]. Nevertheless, they used similar surface chemistry, as they had nanodiamonds with carboxyl moieties and in this study, oxygen terminated nanodiamonds were used[11]. Therefore, although size does not seem to matter much, oxygen terminated surface chemistry could be important for HUVEC cell up take of nanodiamonds. The abilities for MDA-MB-231 cells to take up nanodiamonds also have been studied before. These were smaller than those used here, specifically 45 nm[13]. However, they were manufactured similarly through a high-pressure high-temperature (HPHT) process. Woodhams et al. compared graphitic and oxidized nanodiamonds, and the magnitude in which MDA-MB-231 cells take these nanodiamonds up[13]. This led to the results that oxidized nanodiamonds were much more abundant in the cells than graphitic, thus showing that oxygen terminated nanodiamonds were able to enter MDA-MB-231 cells[13]. Again, surface chemistry seemed more important than size in the capability of cells to take up nanodiamonds. Similar migration studies with nanodiamonds have been conducted on a range of cell types, with of course a range of results. For instance, Guo et al. has conducted migration experiments on HeLa and B16 cells, with homemade carboxylated HPHT nanodiamonds of multiple sizes, namely 30 nm, 50 nm, and 100 nm[19]. They concluded that nanodiamonds inhibit migration, with stronger effects in higher concentrations. Additionally, they noted that the smaller the particles, the more migration inhibition was observed[19]. This was confirmed by Wierzbicki et al., they studied U87 and U118 glioblastoma cell lines in combination with 2-7 nm detonation nanodiamonds, administered in a dose of 50 µg/ml[20]. However, Wierzbicki et al. used detonation nanodiamonds, and in this study HPHT nanodiamonds were used. Since these two types of nanodiamonds are very different in their properties, some of the discrepancies between these two studies can be explained. Additionally, Gao et al. showed that administering HPHT nanodiamonds to cells could also lead to increased cell migration[7]. They studied

four cell types, specifically HeLa, C6, MDCK, and NIH/3T3, in combination with multiple concentrations of carboxylated 100 nm nanodiamonds. In the C6 and NIH/3T3 cells they also observed decreased migration with increased nanodiamond concentration, similar to Guo et al. and Wierzbicki et al., and the MDA-MB-231 cells shown in this paper[7, 19, 20]. Nevertheless, Gao et al. observed migration increase with lower concentrations in HeLa and MDCK cells, and decrease in the higher concentrations for those cells, akin to the observations made in HUVECs in this study. They speculated that alteration of cell migration ability is affected by capacity of nanodiamond uptake of the cells, with the higher the uptake, the lesser cells migrate[7]. Thus, the differences in nanodiamonds effects on migration between the cell types in this study and in other studies could be due to the size of the nanodiamonds and the uptake capability of the cell types. Furthermore, different cell types are naturally inherently different, and may therefore also have different base migration speeds, resulting in differing alterations in migration speed by nanodiamonds.

In order to solidify this study, some steps can be taken. For instance, it would be interesting to see what effect a concentration of 100 µg/ml on the MDA-MB-231 cells would have, and to zoom in on the later timepoints to determine exactly when the differences become significant. Furthermore, it would be useful to have more samples for the MDA-MB-231 cell uptake study, as the 4 h and 24 h groups do not contain that many cells. Additionally, the migration study could also benefit from more samples per group, as the standard deviation is quite large. And lastly, some manual correction was needed for analysing the images. This could be due to the low contrast of the images, which resulted in the program not being able to pick up on all cell borders in the migration study. Thus, some human error could be in the results, although the same correction was used for all images, therefore making the possible deviations consistent.

Although this study has shown the effects of nanodiamonds on cell migration in HUVEC and MDA-MB-231 cells, there is plenty more room for research. Firstly, it is essential to know why and how nanodiamonds influence cell migration. This could be studied for example by focusing on the effects of nanodiamonds on focal adhesion points, or on cytoskeleton alterations. Furthermore, there are plenty of cell types which should be observed for the effects of nanodiamonds before these can be used in migration studies, since the effects are distinctive per cell type. When the preliminaries of using nanodiamonds in migration studies are done, a whole new way of doing migration research could be achieved. To elaborate, nanodiamonds could be used for long term studies, as they do not bleach[9, 10]. Furthermore, their ability to make optical detection of magnetic resonance possible allows for the observation of free radicals and other magnetically active components and their effect on cell migration. This new method of studying migration could drive our knowledge on cell migration and therefore the corresponding fields of development, wound healing and cancer forward.

Bibliography

1. Norden, C., Lecaudey, V. Collective cell migration: general themes and new paradigms. Current opinion in genetics & development 2019; 57: 54-60.
2. Friedl, P., Gilmour, D. Collective cell migration in morphogenesis, regeneration and cancer. Nature reviews: molecular cell biology 2009; 10: 445-457.
3. Zhang, Y., Xu, G., Lee, R.M., Zhu, Z., Wu, J., Liao, S., Zhang, G., Sun, Y., Mogilner, A., Losert, W., Pan, T., Lin, F., Xu, Z., Zhao, M. Collective cell migration has distinct directionality and speed dynamics. Cellular and molecular life sciences 2017; 74: 3841-3850.
4. Enomoto, U., Imashiro, C., Takemura, K. Collective cell migration of fibroblasts is affected by horizontal vibration of the cell culture dish. Engineering in life sciences 2020; 20: 402-411.
5. Campbell, K., Rossi, F., Adams, J., Pitsidianaki I., Barriga, F.M., Garcia-Gerique, L., Batlle, E., Casanova, J., Casali, A. Collective cell migration and metastases induced by an epithelial-to-mesenchymal transition in *Drosophila* intestinal tumors. Nature communications 2019; 10: 2311.
6. Kong, Y., Wang, H., Lin, T., Wang, S. Sphingosine-1-phosphate/S1P receptors signaling modulates cell migration in human bone marrow-derived mesenchymal stem cells. Mediators of inflammation 2014; 2014: 565369.
7. Gao, G., Liu, R., Guo, Q., Wang, H., Li, J., Wang, Y., Chen, X., Zhi, J. The effect of carboxylated nanodiamonds on tumor cells migration. Diamond & related materials 2020; 105: 107809.
8. Schirhagl, R., Chang, K., Loretz, M., Degen, C.L. Nitrogen-vacancy centers in diamond: nanoscale sensors for physics and biology. Annual review of physical chemistry 2014; 65: 83-105.
9. Van der Laan, K.J., Hasani, M., Zheng, T., Schirhagl, R. Nanodiamonds for in vivo applications. Small 2018; 1703838.
10. Nagl, A., Hemelaar, S.R., Schirhagl, R. Improving surface and defect center chemistry of fluorescent nanodiamonds for imaging purposes – a review. Analytical and bioanalytical chemistry 2015; 407: 7521-7536.
11. Gerstenhaber, J. A., Marcinkiewicz, C., Barone, F. C., Sternberg, M., D'Andrea, M. R., Lelkes, P. I., Feuerstein, G. Z. Biocompatibility studies of fluorescent diamond particles-(NV)~800nm (part V): in vitro kinetics and in vivo localization in rat liver following long-term exposure. International journal of nanomedicinde 2019; 14: 6451-6464.
12. Lukowski, S., Neuhoferova, E., Kindermand, M., Krivohlava, R., Mineva, A., Petrakova, V., Benson, V. Fluorescent nanodiamonds are efficient, easy-to-use cyto-compatible vehicles for monitored delivery of non-coding regulatory RNAs. Journal of biomedical nanotechnology 2018; 14: 946-958.
13. Woodhams, B., Ansel-Bollepalli, L., Surmacki, J., Knowles, H., Maggini, L., De Volder, M., Atatüre, M., Bohndiek, S. Graphitic and oxidised high pressure high temperature (HPHT) nanodiamonds induce differential biological responses in breast cancer cell lines. Nanoscale 2018; 10: 12169-12179.
14. Saha, T., Houshyar, S., Sarker, S. R., Pyreddy, S., Dekiwadia, C., Nasa, Z., Padhye, R., Wang, X. Nanodiamond-chitosan functionalized hernia mesh for biocompatibility and antimicrobial activity. Journal of biomedical materials research part A 2021; 1-12.
15. Şelaru, A., Drăguşin, D. M., Olăret, E., Serafim, A., Steinmüller-Nethl, D., Vasile, E. Iovu, H., Stancu, I. C., Costahce, M., Dinescu, S. Fabrication and biocompatibility evaluation of nanodiamonds-gelatin electrospun materials designed for prospective tissue regeneration applications. Materials 2019; 12: 2933.

16. Hemelaar, S.R., Saspaanithy, B., L'Hommelet, S.R.M., Perona Martinez, F.P., Van der Laan, K.J., Schirhagl, R. The response of HeLa cells to fluorescent nanodiamond uptake. Sensors 2018; 18: 355.
17. Jonkman, J.E.N., Cathcart, J.A., Xu, F., Bartolini, M.E., Amon, J.E., Stevens, K.M., Colarusso, P. An introduction to the wound healing assay using live-cell microscopy. Cell adhesion & migration 2014; 8(5): 440-451.
18. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A. Fiji- an open source platform for biological image analysis. Nature methods 2012; 9: 676-682.
19. Guo, Q., Li, L., Gao, G., Liu, R., Einaga, Y., Zhi, J. Nanodiamonds inhibit cancer cell migration by strengthening cell adhesion: implications for cancer treatment. ACS applied materials & interfaces 2021; 13: 9620-9629.
20. Wierzbicki, M., Jaworski, S., Kutwin, M., Grodzik, M., Strojny, B., Kurantowicz, N., Zdunek, K., Chodun, R., Chwalibog, A., Sawosz, E. Diamond, graphite, and graphene oxide nanoparticles decrease migration and invasiveness in glioblastoma cell lines by impairing extracellular adhesion. International journal of nanomedicine 2017; 12: 7241-7254.

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