Time-Resolved Quantification of Nanoparticle Uptake, Distribution, and Impact in Precision-Cut Liver Slices

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Much effort within the nanosafety field is currently focused on the use of advanced in vitro models to reduce the gap between in vitro and in vivo studies. Within this context, precision-cut tissue slices are a unique ex vivo model to investigate nanoparticle impact using live tissue from laboratory animals and even humans. However, several aspects of the basic mechanisms of nanoparticle interactions with tissue have not yet been elucidated. To this end, liver slices are exposed to carboxylated and amino-modified polystyrene known to have a different impact on cells. As observed in standard cell cultures, amino-modified polystyrene nanoparticles induce apoptosis, and their impact is affected by the corona forming on their surface in biological fluids. Subsequently, a detailed time-resolved study of nanoparticle uptake and distribution in the tissue is performed, combining fluorescence imaging and flow cytometry on cells recovered after tissue digestion. As observed in vivo, the Kupffer cells accumulate high nanoparticle amounts and, interestingly, they move within the tissue towards the slice borders. Similar observations are reproduced in liver slices from human tissue. Thus, tissue slices can be used to reproduce ex vivo important features of nanoparticle outcomes in the liver and study nanoparticle impact on real tissue.

1. Introduction

Advanced models such as co-cultures, 3D models and organon-a-chip devices, are highly sought within the nanomedicine and nanosafety fields to bridge the gap between in vitro and in vivo testing.^[1-6] They could contribute to speeding up the translation of nanomedicines to the clinic, and ideally allow screening of multiple nanomaterials, reducing the need for animal testing. Precision-cut tissue slices cultured ex vivo constitute an interesting alternative to fulfill several of these aspects and are already well-established as useful models for testing the mechanism and toxicity of small compounds and drugs.^[7–10]

Tissue slices of 5 mm diameter and around 250 μ m thickness (roughly ten cell layers) can be prepared from different organs (including diseased organs) and potentially any species, including from humans. They possess the complexity and architecture of real 3D tissue and allow preserving ex vivo features essential for tissue function. In fact, they include all (primary) cells normally present in a tissue, with their natural abundance and organization (Table S1, Supporting

Information summarizes some of the key features of this established model).^[7–9]

Several studies that use tissue slices with nanoparticles have been published.^[11–14] In most cases, slices have been

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used to determine nanoparticle impact on the tissue, but also to test nanoparticle formulations for drug delivery, with many examples focused on the lungs,^[11,13,15–17] but also intestine,^[18] liver,^[1,2] and tumor tissue.^[19] Most of these works used tissue from laboratory animals, with the exception of one study using human tissue from the lungs^[17] and one from intestine.^[18] Additionally, only in few cases the details of the cell types in which nanoparticle uptake was observed was included.^[14] Similarly, detailed studies to quantify nanoparticle distribution within the tissue over time, and to determine uptake kinetics in the different cell types in which nanoparticle accumulate have not been reported yet.

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More generally, a clear understanding of the basic mechanisms of nanoparticle interactions with tissue is missing. Thus, it is not always clear how to connect nanoparticle outcomes on tissue to what is observed in standard cell cultures or, indeed, in vivo. For instance, small molecules typically move relatively freely through tissue, and thus their distribution in vivo is largely captured by a tissue slice submerged in a solution of the compound of interest.^[20] In contrast, nanoparticles are internalized and trafficked by cells using endogenous pathways and usually do not exit the cell again.^[21-23] Thus, when applied in vivo, depending on the route of exposure or administration, they will interact with specific cells, into which they may enter and are likely to remain unless they are able to transcytose or are exported. Penetration into tissue is, in fact, a barrier for nanosized drug carriers.^[23-25] Instead, when a tissue slice is submerged in a dispersion of nanoparticles, these can have access to any of the cells on the outer surface of the slice. This outer layer has a cell composition that depends on the way the slice has been cut. In other words, nanoparticles may directly get in contact with cells which in vivo they may never access. Because of this, it is important to determine whether tissue slices allow to reproduce ex vivo outcomes observed in in vivo nanoparticle distribution studies.

Another important aspect to consider is that liver slices are usually maintained in serum-free medium. However, nano-sized objects when applied in biological environments are modified by adsorption of biomolecules on their surface, and the resulting corona layer strongly affects the subsequent interactions with cells.^[26–28] Thus, when testing nanoparticles, some biological fluid needs to be included to allow corona formation and to avoid unrealistic interactions with bare surfaces. However, it is not yet know how corona effects translate to tissue.

Overall, a better knowledge of nanoparticle behavior and outcomes at tissue level is needed in order to establish how tissue slices can be used to extract meaningful information for nanomedicine and nanosafety studies.

2. Results and Discussion

As a first step, we aimed at comparing the response of the tissue to the outcomes observed in vitro using standard cell cultures and using the same nanomaterials. To this end, we exposed rat liver tissue slices to carboxylate- and amino-modified polystyrene nanoparticles (fluorescently labeled 40 nm PS-COOH and unlabeled 50 nm PS-NH₂). The positively charged PS-NH₂ nanoparticles have been shown to induce cell

death by apoptosis, while the PS-COOH nanoparticles usually persist within the cells without any evident toxicity.^[22,29-31] Thus, these materials were selected as well-characterized models in order to test how mechanisms of toxicity observed on cells translate to tissue. Additionally, to determine potential corona effects in tissue, as a first approximation for liver testing, nanoparticles dispersed in medium with 5% fetal bovine serum (FBS) were tested, together with (artificial) serum-free dispersions. Prior to exposure to the slices, the dispersions of the nanoparticles in media with and without serum were characterized (Figure S1 and Table S2, Supporting Information). For the PS-COOH nanoparticles, dynamic light scattering confirmed formation of stable dispersions in both media, with some agglomeration after 24 h in serum-free conditions. In contrast, for the PS-NH₂ nanoparticles, good dispersions were obtained in serum-free medium, while agglomeration was observed in 5% FBS, suggesting that these nanoparticles are less stable in the presence of serum.

Then, rat liver slices were exposed for up to 72 h to the two nanoparticles in the two media (Figure 1). Different nanoparticle concentrations were used to enable direct comparison of the outcomes with in vitro studies on cells exposed to similar concentrations of the same nanomaterials.^[30,31] The ATP and protein levels were used to assess tissue viability (see Experimental Section for details). For slices exposed to the PS-NH₂ nanoparticles, a strong reduction of slice viability was detected both in the absence or presence of a corona in serum (up to 80% reduction in viability after exposure for 72 h). However, earlier effects were observed for bare nanoparticles in serumfree conditions, where a statistically significant reduction of viability of around 60% was determined after only 24 h exposure to the highest nanoparticle concentration tested (Figure 1a-c and Figures S2-S4, Supporting Information). Morphological analysis of tissue sections after hematoxylin and eosin staining confirmed the viability results and showed loss of tissue structure and strong toxicity in slices exposed to the PS-NH₂ nanoparticles both in the presence and absence of a corona, already after 24 h exposure (Figure 1a). In contrast, consistent with in vitro studies, no significant effects on viability were observed for slices exposed to the PS-COOH nanoparticles under all conditions tested (Figure 1d-f and Figures S2-S4, Supporting Information).^[29]

We next characterized the mechanism of cell death. Cell death by apoptosis can be detected by measuring the activity of the proteases caspase 3 and 7. Similarly, DNA fragmentation, another hallmark of apoptosis, can be imaged by TUNEL assay (see Experimental Section for details). For PS-COOH nanoparticles, activation of caspases was observed, more evident after 72 h exposure in medium with FBS, by which time it roughly doubled in comparison to untreated slices (Figure 1g-i and Figure S5, Supporting Information). However, the TUNEL assay did not show apoptosis activation (Figure S6, Supporting Information). For the PS-NH₂ nanoparticles, instead, around 2.5-fold increase in caspase activation was observed already after 24 h exposure, both in 5% FBS (for which no decrease of slice viability had been detected yet) and in serum-free medium (Figure 1g). TUNEL imaging confirmed the presence of many apoptotic cells (Figure 1g). Apoptosis activation was less evident for increasing exposure times, probably due to the strong



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Figure 1. Viability and apoptosis in rat liver slices exposed to PS-NH₂ and PS-COOH nanoparticles. Liver slices were exposed for 24 h to increasing doses of a) PS-NH₂ and d) far-red PS-COOH nanoparticles in serum-free medium (WME) or medium supplemented with 5% FBS (WME + 5% FBS). Additionally, slices were exposed to 100 μ g mL⁻¹ nanoparticles in the two media for 48 and 72 h b,c) for PS-COOH, and e,f) for PS-NH₂, respectively. Viability is expressed as the ATP content (pmol) normalized by total protein amount (μ g). Tissue morphology after 24 h exposure to 100 μ g mL⁻¹ nanoparticles was assessed by histochemistry ((a) and (d), for cross-sections of slices exposed to PS-COOH and PS-NH₂ nanoparticles, respectively). Scale bar 200 μ m. Caspase 3/7 activity was also measured in slices exposed for g) 24, h) 48, and i) 72 h to 100 μ g mL⁻¹ nanoparticles in the two media. The viability and caspase data show the mean and standard error of the mean (SEM) of the results obtained from three independent experiments. Every dot represents the result of an independent experiment. For each experiment, the results of the treated slices are compared to the results of untreated control slices from the same animal (0 μ g mL⁻¹). For each condition three slices of the same animal were used, and the average and SEM were calculated (the individual viability experiments are shown in S2–S4, Supporting Information and the raw caspase results in Figure S5, Supporting Information). Confocal fluorescence images of TUNEL assay on cross-sections of untreated control slices cultured for 24 h and slices exposed for 24 h to 100 μ g mL⁻¹ PS-NH₂ in serum-free medium are also included to identify potential apoptotic cells (g). Blue: DAPI-stained nuclei. Green: TUNELpositive nuclei of apoptotic cells. Scale bar: 100 μ m. Exposure to PS-NH₂ nanoparticles leads to decreased viability in both media and activation of apoptotic cells. Scale bar: 100 μ m. Exposure to PS-NH₂ nanoparticles leads to decreased viability in

reduction in viability under these conditions (Figure 1h,i). Similar outcomes were also observed with murine liver slices exposed to the two nanoparticles (Figure S7, Supporting Information).

Overall, these results indicate that the tissue slices responded to the selected model nanoparticles in similar ways as reported for different cell cultures in in vitro studies.^[30,31] However, the activation of caspases in slices exposed to the PS-COOH nanoparticles may be a sign of a different response of the tissue in comparison to what is observed on cells with the same material, for which no sign of apoptosis has been reported.^[29] Similarly, in the case of the PS-NH₂ polystyrene, usually, bare positive nanoparticles exhibit much stronger toxic effects on cells than corona-coated ones.^[31] while here the difference was rather small. This is likely a specific effect due to tissue characteristics in comparison to cell cultures. Thus, while in a standard cell culture every cell comes immediately into contact with the nanoparticle dispersion, in the tissue slice only the outer layer of cells (initially) interacts with the nanoparticles and hence one would expect a less evident difference in the impact of the bare and corona-coated nanoparticles. Nevertheless, it is clear that the effect of a corona should be considered also in tissue and, especially when using liver slices, artificial serum-free conditions should be avoided.

As a next step, it will be important to perform similar studies exposing tissue to nanoparticle concentrations comparable to those to which the liver is exposed in vivo. These are hard to determine and will vary depending on the nanoparticle and application investigated. As an example, in order to obtain some preliminary information on likely concentrations to which the liver may be exposed, we have compared our conditions with the results reported by Ogawara et al^[32] with similar polystyrene nanoparticles. In their work, 50 nm polystyrene similar to those used in our study were injected IV in rats at a dose of 12.5 mg kg⁻¹ and the authors found that around 50% of the injected dose accumulated in the liver. This roughly corresponds to an initial blood concentration of 200 μ g mL⁻¹ nanoparticles, comparable to the concentrations used here.

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Next, in order to understand more in detail the observed impact at tissue level (Figure 1), we turned to resolving whether nanoparticles were internalized by cells in the tissue ex vivo. More specifically, we aimed at determining the cell types involved both in uptake and—as a consequence of uptake—in the toxic responses observed in slices exposed to the PS-NH₂ nanoparticles. After optimization to overcome the natural autofluorescence of the tissue and reduce the interference of extracellular nanoparticles adhering onto the outer surface of the slice (Figure S8, Supporting Information), confocal fluorescence imaging of rat liver slices exposed to fluorescently labeled PS-COOH nanoparticles clearly confirmed nanoparticle uptake into cells of the tissue (Figure 2 and Figure S9, Supporting Information). Images of transversal sections of the slices showed that, as expected, the nanoparticles were mainly internalized by cells located in the outer layer, though some nanoparticles were observed in cells deeper into the tissue as well. One could expect nanoparticles to be internalized by any of the cell types present in the outer layer of the slice, with the hepatocytes (roughly 70% of all liver cells)^[33,34] presumably being highly prevalent. Instead, imaging showed that some cells accumulated more nanoparticles. Immunostaining clearly indicated that these were the liver Kupffer cells (Figure 2a-c; overlap of red and green signal).^[32,35] Uptake in CD-31 stained vascular and lymphatic endothelial cells or SE-1 stained liver sinusoidal cells was observed only in rare cases, in slices where these cell types were present in the outer cell layers (Figures S10 and S11, Supporting Information). A preferential uptake in Kupffer cells was also observed on murine liver slices exposed to the same nanoparticles (Figure S12, Supporting Information).

It is important to compare these observations with in vivo intrahepatic distribution studies. As an example, using polystyrene microsphere of 50 and 500 nm, Ogawara et al^[32] found that after IV injection, within the liver 60-70% of the particles were taken up by Kupffer cells. However, uptake in parenchymal and endothelial cells was also present (28% and 13%, respectively, for the smaller particles and 5% and 24% for the larger ones). Similar results were obtained on mice by Park et al^[36] with PLGA particles, which were taken up by Kupffer cells with highest efficiency, although uptake was present also in liver sinusoidal cells and hepatic stellate cells, and-to much lower extent-in hepatocytes. Similar results were reported by Dragoni et al^[14] after injection of gold nanoparticles in rat. Lee et al.^[37] compared the distribution of silica nanoparticles with different surface functionalization within the liver after IP injection in mice. They also found the highest accumulation in the Kupffer cells. However, uptake in liver sinusoidal cells was also comparable. On the contrary, Sadauskas et al^[38] studied the distribution of gold nanoparticles within the liver in mice after IV and IP injection and found uptake only in Kupffer cells.

Overall, these studies indicate that within the liver in most cases the Kupffer cells show higher accumulation of nanoparticles. Importantly, our results show that, even though nanoparticles enter the tissue in a very different way in comparison to arrival from the blood vessels, tissue slices mimic this key feature of in vivo distribution studies, namely a preferential nanoparticle accumulation in Kupffer cells.

We also noted a higher intensity of ED2 staining in comparison to slices not exposed to the nanoparticles (Figure 2, also visible in the single channel images of **Figure 3**). ED2, also known as CD163, is a surface glycoprotein, whose expression increases in the context of resolution of inflammation and tissue repair.^[39,40] The higher expression of this marker suggests that the Kupffer cells maintain their key functions in the tissue slice and respond to nanoparticle exposure. This is an interesting observation, which we analyzed in detail in a separate study (Bartucci et al, in preparation).

As a next step, we attempted to connect the response to the PS-NH₂ nanoparticles at tissue level (the activation of apoptosis and decreased tissue viability, as shown in Figure 1) with the observed nanoparticle uptake and distribution within the tissue. To this end, we combined a TUNEL assay to detect apoptotic cells with immunostaining by ED2 to identify the Kupffer cells (Figure 2d). Kupffer cells engulfing apoptotic cells to clear them—if present—can also be stained by TUNEL assay.^[41] However, imaging clearly showed that in the slices exposed to the PS-NH₂ nanoparticles, most of the TUNEL positive cells were ED2-positive. These results suggest that the apoptotic cells were mainly Kupffer cells. Thus, tissue slices can be used to connect the response at tissue level to the effect nanoparticles induce in the specific cell types in which they accumulate.

We then followed nanoparticle uptake and distribution within the tissue for up to 72 h (Figures 2,3 and Figures S13-S15, Supporting Information). With increasing exposure time, naturally, nanoparticle uptake increased (Figure 2). Interestingly, we observed accumulation of Kupffer cells at the slice borders, both for control and nanoparticle-exposed slices (Figure 3a-c). It is known that Kupffer cells have the capacity to move within the tissue: for instance infiltration of Kupffer cells was observed in liver in animals exposed to titania nanoparticles.^[42] Similar effects were also observed in other studies with liver slices.^[43] In order to investigate if this effect was due to the nanoparticles, we quantified it by calculating the (closest) distance of each Kupffer cell from the slice border. Thus, Figure 3d shows the fraction of Kupffer cells identified in each slice as a function of their distance from the slice borders, including data from all slices under the same conditions. This gives the distribution of Kupffer cells in untreated and PS-COOH treated slices, but is confounded at larger distances by the unequal sizes of the imaged slices. It may be observed that already after 24 h exposure to PS-COOH nanoparticles, a substantial fraction of the Kupffer cells had moved to the slice border (within 20 µm from it) (Figure 3d). This accumulation is clear also after 48 and 72 h (also in Figure 3d). There is some accumulation at the border also for the Kupffer cells in control slices not exposed to the nanoparticles (Figure 3d), in comparison to what is observed in a fresh slice of liver tissue (Figure S16,

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Figure 2. Confocal fluorescence imaging of rat liver slices exposed to PS-COOH and PS-NH₂ nanoparticles. Cross-sections acquired by confocal fluorescence microscopy of liver slices exposed to $10 \,\mu\text{g} \,\text{mL}^{-1}$ far-red PS-COOH nanoparticles in 5% FBS medium for a) 24, b) 48, and c) 72 h and d–f) to 50 $\mu\text{g} \,\text{mL}^{-1}$ (unlabeled) PS-NH₂ nanoparticles in 5% FBS medium for 24 h. Indicated areas are shown at increased magnification to the right (a–c). Scale bars, from the left: 100, 40 and 10 μ m. Blue: DAPI-stained nuclei. Red: nanoparticles. Green: ED2-labeled Kupffer cells. The results confirm nanoparticle uptake into the tissue and, interestingly, high nanoparticle uptake by Kupffer cells, mainly at the edge of the tissue slices, as evidenced by the overlap between the nanoparticle (red) and Kupffer cell label (green) signal. A TUNEL assay was performed on slices exposed to PS-NH₂ nanoparticles in order to stain apoptotic cells (d–f) (see Experimental Section for the details). Blue: DAPI-stained nuclei. Red: ED2-labeled Kupffer cells. Green: apoptotic (TUNEL positive) cells. The indicated region in panel d is shown at increased magnification in panel e, and similarly for panels (e,f). Scale bar: d) 100, e) 50, and f) 10 μ m. Imaging showed that most of the apoptotic cells (green) were also ED2-labeled (red) and there were not many apoptotic cells that were not ED-2 labeled. This all together suggests that in the slices exposed to the nanoparticles most apoptotic cells were Kupffer cells (as indicated by the substantial overlap of the apoptotic (green) and Kupffer cell (red) signals) accumulated at the slice borders.



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Distance (µm)

Figure 3. Quantification of Kupffer cell distribution within rat liver slices at increasing exposure times. Liver slices were exposed to $10 \mu \text{g mL}^{-1}$ far-red PS-COOH nanoparticles in 5% FBS medium for a) 24, b) 48, and c) 72 h prior to confocal imaging and quantification of Kupffer cell distribution within the tissue, performed as described in the Experimental Section. Representative cross-sections acquired by confocal fluorescence microscopy of liver slices with ED2-stained Kupffer cells (a–c). Untreated control slices cultured for the same time in medium with 5% FBS without nanoparticles (untreated) and slices exposed to the nanoparticles (PS-COOH treated) are both shown. Scale bar: $200 \,\mu$ m. The signal of the Kupffer cell distribution within the slice, expressed as the fraction of Kupffer cells as a function of the distance from the slice borders (see Experimental Section for details), for untreated tissue slices (top panels, blue bars) and slices exposed to PS-COOH nanoparticles (bottom panels, red bars). The results show accumulation of Kupffer cells exposed conditions, with some accumulation toward the borders also for control slices. Furthermore, slices exposed to the nanoparticles seem to have higher ED2 expression, suggesting Kupffer cell activation. Counted Kupffer cells for the 24 h control, 305; 48 h control, 219; 72 h control, 781; 24 h treated, 283; 48 h treated, 208; 72 h treated, 790 Kupffer cells. e) The proportion of Kupffer cells within the first 20 μ m for all slices investigated, normalized by that expected due to chance (see Experimental Section for details). The results for untreated control slices are shown in blue and those for slices seposed to the nanoparticles within the first 20 μ m for all slices investigated, normalized by that expected due to chance (see Experimental Section for details). The results for untreated control slices are shown in blue and those for slices exposed to the nanoparticles in red with data points indicating individual slices, the bar the mean o



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Figure 4. Flow cytometry analysis of nanoparticle uptake by Kupffer cells recovered from murine liver slices exposed to PS-COOH nanoparticles. Liver slices were exposed to 25 µg mL⁻¹ far-red PS-COOH nanoparticles in 5% FBS medium for different times up to 48 h prior to tissue digestion, performed as described in the Experimental Section. The isolated cells were analyzed by flow cytometry. a) Fraction of cells with nanoparticles, determined as the number of cells with nanoparticles normalized by the total number of cells measured. b) Fraction of Kupffer cells with nanoparticles, determined as the number of CD68 positive cells with nanoparticles normalized by the total number of CD68 positive cells. The fraction of cells with nanoparticles increases with increasing exposure time. The high percentage of Kupffer cells with nanoparticles is consistent with the accumulation of Kupffer cells toward the slice borders observed by microscopy. c) Mean cell fluorescence intensity due to nanoparticle uptake of the fraction of cells with nanoparticles. The mean fluorescence intensity over time of the Kupffer cells (CD68 positive cells) with nanoparticles and all other cells (CD68 negative cells) with nanoparticles are shown. In a-c the average and standard deviation of the results obtained in three independent experiments are shown (with the exception of the 16 h sample performed only in two experiments). For each condition 20 000-70 000 individual cells were acquired (see Experimental Section for details). Uptake increases over time in both Kupffer cells and all other cells with nanoparticles, but at all times the average intensity of Kupffer cells with nanoparticles is higher than that of all other cells with nanoparticles. This suggests that Kupffer cells are the cells with highest uptake efficiency. d) Representative double scatter plots of cell fluorescence intensity in the nanoparticle channel (nanoparticle uptake, PS-COOH) versus CD68 staining. The Kupffer cells (CD68 positive) are shown in red and all other cells (CD68 negative) are shown in green. The results show that as time increases more cells internalize nanoparticles (as indicated by the increasing number of dots inside the rectangular gate) and a population of cells with much higher nanoparticle uptake becomes visible (delimitated by a round gate). CD68 staining confirms that most of these cells are Kupffer cells (red CD68 positive dots, see also Figure S21, Supporting Information for further analysis).

Supporting Information). This can be connected to the initial response of the tissue to the damage caused by the cutting procedure. However, the effect is clearly stronger for slices exposed to the nanoparticles. Comparison of the distributions for individual slices shows that accumulation at the border is prevalent in some slices, but not all slices exhibit this behavior (Figures S17–S19, Supporting Information). This is likely a consequence of the high heterogeneity in Kupffer cell distribution within the liver^[44] and variability across animals as well as tissue slices. As a further assessment of the effect, we quantified the proportion of Kupffer cells within the first 20 µm, also normalizing by the proportion expected due to chance for each slice (see Experimental Section for details). The proportion of cells within the first 20 µm was higher for the slices exposed to PS-COOH nanoparticles at all times (Figure 3e). Accumulation of Kupffer cells toward the slice borders was observed also with unlabeled PS-COOH nanoparticles and the toxic unlabeled PS-NH₂ model (Figure S20, Supporting Information), thus excluding artefacts due to nanoparticle fluorescence spillover. Overall, these results suggest that, as observed in vivo,^[42] the primary Kupffer cells in the tissue slices retain their capacity to respond to signals and accumulate toward the site of exposure to—in this case—the nanoparticles.

Next, as a further confirmation of what was observed by confocal microscopy and in order to quantify nanoparticle uptake kinetics in the Kupffer cells and all other cells, murine liver slices exposed to PS-COOH nanoparticles were digested enzymatically (see Experimental Section for details). Enzymatic digestion opens up the possibility to recover mixtures of all cells from the tissue after exposure to the nanoparticles, and perform further analysis and quantitative studies at individual cell level.

Following enzymatic digestion, we used flow cytometry to measure nanoparticle uptake in several thousand individual cells recovered from the tissue over time. Combined with immunostaining to identify the Kupffer cells (Figure S21, Supporting Information), this allowed us to determine nanoparticle uptake kinetics in these and all other cells (**Figure 4**). The results clearly showed that with increasing exposure time the fraction of cells containing nanoparticles increased (Figure 4a), and, interestingly, after 48 h 80% of the Kupffer cells contained nanoparticles (Figure 4b). This is consistent with the accumulation of Kupffer cells toward the slice borders observed by fluorescence microscopy (Figures 2,3). Furthermore, as suggested by imaging, flow cytometry analysis confirmed that in all cells with nanoparticles uptake increased over time, but at all times the Kupffer cells were the cells with the highest nanoparticle fluorescence (Figure 4c–d). These results confirmed once more that tissue slices allow to resemble ex vivo key features of what is observed in vivo in the liver, and, more specifically, a preferential accumulation of nanoparticles by Kupffer cells, as well as the capacity of these specialized cells to respond to stimuli and accumulate toward the sites of exposure.

Finally, similar studies were performed using human (as opposed to rat and murine) liver tissue slices isolated from surgical waste material. To the best of our knowledge this is the first study using live human liver tissue to test nanoparticle uptake and impact. The results (**Figure 5** and Figures S22 and S23, Supporting Information) show that similar outcomes could be observed also in human liver tissue. Thus, exposure to the PS-NH₂ nanoparticles led to a decrease in viability (Figure 5a–b) and, also in human liver slices, PS-COOH nanoparticles were taken up preferentially by Kupffer cells at the slice borders (Figure 5e,f). This opens up the possibility to use this model to study nanoparticle uptake and impact on primary human Kupffer cells still embedded in their normal tissue environment.

3. Conclusions

In summary, in this work we investigated the basic mechanisms of nanoparticle interactions with liver tissue slices in order to understand how the outcomes observed in standard cell cultures and in vivo translate to tissue. To this end, we used slices from rat and murine livers and-for the first time for nanoparticle studies-also from human liver. We combined time resolved confocal microscopy and flow cytometry on individual cells recovered from the tissue to perform a quantitative study of nanoparticle distribution and uptake in the different cell types in which they accumulate within the tissue. As a first step, tissue slices were exposed to doses comparable to in vitro studies performed on standard cell cultures with the same nanomaterials, thus allowing a direct comparison of the outcomes in the tissue slices to those obtained on simpler cell culture systems. Comparable outcomes were, indeed, observed in tissue slices, including effects related to the presence or absence of a corona on the nanoparticles. As a future step, it will be important to repeat similar studies with doses comparable to those to which the liver may be exposed in vivo. This is hard to determine and of course will depend on each specific nanoparticle and condition investigated. For instance, in the context of nanosafety, the dose that arrives to the liver will depend (among many other factors) on exposure, while in the context of nanomedicine one may refer to administered doses and clinically relevant doses. Nevertheless, most nanoparticle distribution studies show preferential particle accumulation in the liver, thus there is little doubt on the likely exposure of the liver to nanoparticles.

While the advantages of using real tissue, including from humans, are clear, tissue slices, like every model, also present some limits. One is the relatively short viability, currently optimized to up to around 5 days after excision.^[43] Further optimization could include, for instance, the addition of a microfluidic system, which could also be used to connect slices from different organs.^[45] Perhaps the major limitation in the use of tissue slices is related to the way nanoparticles are exposed to the tissue and come into contact with the different cell types within this organ in comparison to exposure from the blood in vivo. For instance, uptake in liver sinusoidal cells was observed mainly when these cells were present in the outer cell layer of the tissue slices. Thus, the model is likely to underestimate specific effects on liver sinusoidal cells for nanoparticles which show substantial uptake in this cell type in vivo.^[36,37] Nevertheless, in most cases intrahepatic distribution studies have shown that the cells with highest uptake efficiency within the liver are the Kupffer cells^[14,35-38] and our results clearly show that liver tissue slices do resemble ex vivo this key feature. Furthermore, by maintaining them in their tissue environment, these primary macrophages seem to preserve intercellular communication and cell signaling, as suggested by the observed capacity to migrate toward the sites of exposure, to-in this case-the nanoparticles. This indicates that even after tissue extraction, these primary cells maintained some of the key features for their functions and possibly were able to respond to extracellular signals. Thus, after exposing the complete tissue to nanoparticles, fluorescence microscopy and other imaging methods can be used to visualize and characterize Kupffer cells still embedded in their environment, while tissue digestion can be used to recover them from the tissue after exposure. In this way, high throughput quantitative methods, such as flow cytometry used here, can be utilized for a more in depth study at individual cell level. Additionally, by using human samples, tissue slices can be used to gain important insights on possible outcomes of nanoparticles in humans. Overall, this makes tissue slices an attractive ex vivo model for studying the response induced by nanoparticles in the liver and, more specifically, effects induced in primary Kupffer cells, crucial players in nanosafety and nanomedicine outcomes.

4. Experimental Section

Animals: Adult male Wistar rats (250–300 g) and male C57BL/6J mice aged 8—10 weeks were obtained from Harlan Laboratories B.V. Rats and mice were kept in a temperature and humidity-controlled room with a 12 h light/dark cycle with food and water ad libitum. Animals were allowed to acclimatise for at least 1 week before starting the experiments. Rats and mice were sacrificed under isoflurane/O₂ anesthesia and their livers were harvested. The organ was kept in ice-cold University of Wisconsin (UW) organ preservation solution (DuPont Critical Care) until the start of the slicing procedure. All experiments were approved by the Animal Ethical Committee of the University of Groningen.

Human Liver Tissue: The samples of human liver used in the present study were obtained anonymously from patients undergoing partial hepatectomy for the removal of carcinoma (two samples) or from liver tissue remaining as surgical waste after reduced-size liver transplantation (TX) (one sample). The use of human tissue was approved by the Medical Ethical Committee of the University Medical Centre Groningen (UMCG), according to Dutch legislation and the Code of Conduct for dealing responsibly with human tissue in the context of health research (www.federa.org), refraining from the need www.advancedsciencenews.com





Figure 5. Nanoparticle exposure in human liver slices. Human liver slices (prepared as described in the Experimental Section) were exposed for 24 h to increasing doses of a) PS-NH₂ nanoparticles or c) 100 μ g mL⁻¹ PS-COOH nanoparticles in serum-free medium (WME) or medium supplemented with 5% FBS (WME + 5% FBS). Additionally, slices were exposed to 100 μ g mL⁻¹ PS-NH₂ and PS-COOH nanoparticles in the two media for 48 h (b) and (d), respectively). Viability is expressed as the ATP content (pmol) normalized by total protein amount (μ g). The viability data show the mean and standard error of the mean (SEM) of the results obtained from three independent experiments. Every dot represents the result of an independent experiment. For each experiment, the results of the treated slices are compared to the results of untreated control slices from the same animal (0 μ g mL⁻¹). For each condition three slices of the same animal were used, and the average and SEM were calculated (the individual viability experiments are shown in Figures S22 and S23, Supporting Information). Exposure to PS-NH₂ nanoparticles leads to decreased viability in both media. Confocal fluorescence images of cross-sections of slices exposed to 100 μ g mL⁻¹ far-red PS-COOH in 5% FBS medium for e) 24 and f) 48 h are also included. Details of the same area at increasing magnification are shown (scale bars: 100, 20 and 10 μ m, respectively). Blue: DAPI-stained nuclei. Red: nanoparticles. Green: CD68-stained Kupffer cells. Imaging shows that also in human liver slices nanoparticles are internalized efficiently by Kupffer cells accumulating at the slice borders. A Friedman test with Dunn's correction was performed when comparing multiple groups, and Wilcoxon test when comparing two groups. $p \le 0.05$ was considered significant. * = $p \le 0.05$.

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of written consent for "further use" of coded-anonymous human tissue. The procedures were carried out in accordance with the experimental protocols approved by the Medical Ethical Committee of the UMCG.

The samples were perfused with a cold UW organ preservation solution immediately after collection. Fewer conditions could be tested simultaneously for human liver samples in comparison to the experiments performed with rat liver because of the limited sample size available.

Liver Slices Preparation: Precision-cut liver slices were prepared as described previously.^[7] Briefly, rat liver cores were extracted with a drill equipped with a 5 mm diameter cylindrical tip and mouse liver cores were made using a biopsy puncher with a diameter of 5 mm. Afterward, cores were sliced with Krumdieck Tissue Slicer MD6000 (Alabama R&D) filled with ice-cold Krebs–Henseleit buffer supplemented with 25 mm D-glucose (Merck), 25 mm NaHCO₃ (Merck), 10 mm HEPES (MP Biomedicals) and saturated with a mixture of 95% oxygen and 5% CO₂. After the slicing procedure, liver slices of about 200–250 μ m thickness and 5 mg weight were preserved in the UW organ preservation solution on ice until further use.

Pre-Incubation: The liver slices were transferred to a petri dish containing William's Medium E + GlutaMAX (WME, with L-glutamine, Invitrogen) medium supplemented with 25 mmD-glucose and 50 μ g mL⁻¹ gentamycin (Invitrogen) in order to remove the UW solution before the experiments (this medium is referred to as "WME medium" or simply "medium" in the rest of the manuscript). Then the liver slices were transferred to individual wells in a 12-well plate filled with 1.3 mL pre-warmed (37 °C) serum-free WME medium or WME medium supplemented with 5% v/v FBS (Gibco from TermoFisher Scientific), and saturated with 80% $O_2/5\%$ CO_2 . Finally, the slices were maintained in an incubator (Panasonic) at 37 °C saturated with 80% O2/5% CO2 and gentle shaking for 3 h, prior to exposure to the nanoparticles. The 3 h pre-incubation under these conditions allows the tissue to restore its function and decreases the presence of residual cell debris present on the edge of the slices after the cutting procedure, which could affect the subsequent exposure to nanoparticles.

Dynamic Light Scattering Measurement: Far-red labeled 40 nm carboxylated polystyrene nanoparticles (FluoSpheres, PS-COOH, maximum excitation at 660 nm and emission at 680 nm) were purchased from Thermo Fisher Scientific and unlabeled 50 nm amino-modified polystyrene nanoparticles (PS-NH₂) were purchased from Bangs Laboratories. The nanoparticle dispersions in relevant buffers were characterized by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd). Briefly, PS-COOH and PS-NH2 nanoparticles were dispersed in Milli-Q water, Phosphate-Buffered Saline (PBS), serum-free WME medium or WME medium supplemented with 5% v/v FBS by dilution of the nanoparticle stocks to a final concentration of 100 μg mL⁻¹ and measured immediately after dispersion. Additionally, dispersions in serum-free WME medium or WME medium supplemented with 5% v/v FBS were also measured after 24 h incubation in the conditions used for tissue maintenance (37 °C saturated with 80% $O_2/5\%$ CO₂, and gentle shaking). The results are the average of three separate measurements, each containing 10 runs of 10 s.

Exposure to Nanoparticles: After 3 h pre-incubation, the liver slices were exposed to the nanoparticles by transferring them to prewarmed (37 °C) and pre-saturated (80% O₂/5% CO₂) wells containing nanoparticle dispersions at different doses (0–100 μ g mL⁻¹) in serumfree WME medium or WME medium supplemented with 5% v/v FBS, prepared as described above. Then the liver slices were maintained as described above for 24, 48, and 72 h exposure.

Tissue Viability: The ATP content normalized by the total protein content was used as a measure of tissue viability after exposure to nanoparticles. For each experimental condition, three slices were used as replicates. The slice ATP content was determined via an ATP assay as follows: after exposure to nanoparticles, the liver slices were washed twice with medium and once with PBS in order to remove debris and reduce the presence of nanoparticles adhering to the outer tissue layer. Each individual slice was then collected in 1 mL of sonication solution, containing 70% v/v ethanol and 2 mm EDTA (pH 10.9), snap frozen in liquid nitrogen and stored at -80 °C until further analysis. Then the ATP content was measured as described previously. Briefly, samples were thawed slowly on melting ice, homogenized for 45 s using a Mini-BeadBeater 24 (Biospec Products), and centrifuged at 16 100 rcf for 5 min at 4 °C. The supernatant was diluted ten times in 0.1 \times Tris HCl buffer (pH 7.8) containing 2 mM EDTA and the ATP content was determined using the ATP Bioluminescence Assay Kit CLS II (Roche), according to the manufacturer's protocol. Samples were transferred into a black 96-well plate and luminescence was measured using a Lucyl luminometer (Anthos).

The pellet obtained after centrifugation of the homogenised tissue slices was used for determining the slice's total protein content. The pellet was reconstituted in 200 μ L 5 M NaOH for 30 min at 37 °C. After dilution with 800 μ L Milli-Q water, the protein content was measured by a Lowry assay using a Bio-Rad DC Protein Assay (Bio-Rad) following the manufacturer's instructions. Serial dilutions of bovine serum albumin were used to make a calibration curve. Samples were transferred into a black 96-well plate, and after keeping the plate in the dark for 15 min, the absorbance at 650 nm was read using a Molecular Devices Thermo Max Microplate Reader.

Finally, for each slice the viability was obtained by normalizing the ATP value (pmol) by the total protein amount (μ g). For each condition, three slices were used, and the average and standard deviation calculated. Figure 1, Figures S2–S4, Supporting Information for rat liver slices, and Figure 5, Figures S22 and S23, Supporting Information for human liver slices, show the results obtained in three independent experiments (3 animals or 3 human liver samples). Additionally, Figures 1,5 show the average and standard error of the mean of the results obtained in the same 3 independent experiments.

Caspase-Glo 3/7 Assay: Caspase 3/7 activity in the tissue slices was measured using the Caspase Glo 3/7 (Promega), following the procedure previously described to measure Caspase 9 activity.^[46] Briefly, slices were washed twice with serum-free WME medium or WME medium supplemented with 5% v/v FBS, as used for exposure to nanoparticles. For each condition, three samples (three slices) were prepared and, after exposure to nanoparticles, the slices were collected together in a safe-lock vial containing 600 μ L serum-free WME medium. The samples were homogenized immediately using a Mini-Bead Beater for 45 s and centrifuged for 2 min at 4 °C, 16 100 rcf. Then, the supernatant was used to measure caspase activity in 3 separate wells as follows: 5 µL supernatant was transferred into one well of a black 96-well plate (Costar, Corning) with 40 µL Caspase-Glo 3/7 Reagent and 55 µL serum-free WME medium. Subsequently, the plates were gently shaken for 2 min, incubated for 30 min at room temperature in darkness and the luminescence was measured using a luminescence plate reader (LumicountTM). The average and standard deviation of 3 replicate wells were then calculated. Figure S5, Supporting Information shows the average and standard error of the mean of the results obtained in 3 independent experiments. Figure 1 shows the same results after normalization of the values obtained in slices exposed to the nanoparticles with the values obtained in untreated control slices.

Cryo- and Paraffin-Sections of Liver Slices: In order to prepare sections for fluorescence imaging and histochemistry, after exposure to the nanoparticles the slices were transferred to individual wells of a 12-well plate filled with 1.3 mL pre-warmed and pre-saturated nanoparticle-free WME medium or WME medium supplemented with 5% v/v FBS, as during exposure. Then, slices were maintained in the incubator for a further 3 h in order to reduce the potential presence of nanoparticles adhering to the outside of the tissue, which could confuse imaging results (Figure S8, Supporting Information shows images of slices prepared for microscopy directly after exposure or after 3 h in medium without nanoparticles to illustrate this). After 3 h in medium without nanoparticles, slices were washed twice with medium and once with PBS. To prepare cryoblocks, liver slices were embedded in KP-cryocompound (Klinipath) and frozen in 2-methylbutane (Sigma-Aldrich) on dry ice. Afterward, transversal sections of the tissue slices with a thickness of 4 μ m were prepared using a CryoStar NX70 cryostat (Thermo Fisher Scientific) perpendicular to the surface of the slice.

For paraffin embedding, slices were fixed in 4% formaldehyde in PBS for 24 h at 4 °C and stored in 70% ethanol at 4 °C until analysis. After dehydration in alcohol and xylene, the slices were embedded in paraffin and 4 μ m sections were cut perpendicular to the surface of the slice using Leica Reichert-Jung 2040 Autocut Microtome.

Immunofluorescence Staining of Cryo-Sections: Cryo-sections with a thickness of 4 μ m were cut and stained the same day. Sections were dried for 30 min at room temperature, fixed with 4% formaldehyde (Klinipath) for 15 min at room temperature and permeabilized with 0.2% triton X-100 (Sigma-Aldrich) for 15 min. Then, sections were incubated with 50 μ L of primary antibodies for 60 min at room temperature, followed by incubation with secondary antibodies for further 60 min at room temperature in darkness. Antibodies were diluted in 5% serum of the same species (rat, mouse, or human) in PBS in order to block non-specific binding. The primary antibodies used for rat liver slices were: a goat anti-CD163 rat antibody (also known as ED2, AbD SeroTec, 1:50 dilution) to stain Kupffer cells, a mouse anti-CD31 rat monoclonal antibody (BD Biosciences 1:100 dilution) as a general marker for vascular and lymphatic endothelial cells, and mouse anti-SE-1 rat monoclonal antibody (Novusbio, 1:100 dilution) as a more specific marker for hepatic sinusoidal endothelial cells. A mouse anti-CD68 human monoclonal antibody (DAKO, 1:50 dilution) and a rat anti-CD68 mouse monoclonal antibody (BIO-RAD, 1:50) were used to stain Kupffer cells in the human and mouse liver slices, respectively. The secondary antibodies used were a mouse anti-goat Alexa Fluor 555 (Thermo Fisher Scientific, 1:200 dilution), a donkey anti-mouse Alexa Fluor 555 (Thermo Fisher Scientific, 1:200) and a goat anti-rat Alexa 555 (Thermo Fisher Scientific, 1:200).

Cell nuclei were stained by incubation for 5 min in 4',6-diamidino-2-fenylindole (DAPI, 1 μ g mL⁻¹). Finally, the slides were mounted with glass cover slips using MOWIOL 4–88 (Sigma-Aldrich). Images were acquired using a Leica SP8 confocal microscope, using a 40× and 63× objectives, with a 405 nm laser for DAPI excitation, a 552 nm laser for Alexa Fluor 555, and a 638 nm laser for the far-red nanoparticles. For the images shown in Figure 3 and Figure S20, Supporting Information, imaging settings for the nanoparticle and ED2 channels were kept constant in order to allow comparison of the intensity of images of samples at different exposure times.

In order to obtain images of the entire slice section, multiple adjacent images were acquired in the same *z*-plane using the same settings after which individual TIFF files were merged together using the 2D stitching plugin of the Fiji-ImageJ software.

Tissue Digestion and Flow Cytometry: The mouse liver dissociation kit from Miltenyi Biotec was used for the enzymatic digestion of murine liver slices. For each condition, 12 slices were prepared and, after exposure to nanoparticles, were washed for 3 h with WME medium supplemented with 5% v/v FBS. Then, the slices were pulled together in a 50 mL tube containing the dissociation mix, which was prepared as follow: 100 μ L Enzyme D solution, 50 µL Enzyme R solution, and 10 µL Enzyme A solution in 5 mL WME medium supplemented with 5% v/v FBS. The samples were incubated for 15 min at 37 °C in a water bath with shaking and every 5 min samples were gently resuspended. After digestion, the liver tissue was passed through a 70 µm nylon strainer (BD Bioscience) to obtain single cell suspensions and the filters were washed with extra 5 mL of medium. $\approx 1 \times 10^6$ cells per sample were recovered. Cells were centrifuged and resuspended twice in sterile PBS. Then, in order to discriminate live and dead cells, samples were incubated with Fixable Viability Dye eFluo 450 (eBioscience, 1:2000 dilution) in serum/proteinfree PBS for 30 min on ice in the dark. After that, cells were washed twice with a solution of 2% v/v FBS, 5 mm EDTA in PBS (PFE buffer) and incubated with Fix/Perm buffer (eBioscience) for 30 min on ice. Next, cells were washed with Perm-buffer (eBioscience) once. Afterward, the isolated cells were incubated with a PE/Cy7 anti-mouse CD68 antibody (Biolegend, 1:100) for 30 min on ice in the dark in order to stain the Kupffer cells. Finally, cells were washed twice with Perm-buffer, resuspended in PFE buffer, and immediately measured using a Cytoflex Flow Cytometry (Beckman Coulter) with 405 nm (for live/dead staining), 488 nm (for Kupffer cells) and 630 nm (for nanoparticles) lasers. Data

were analyzed using Flowjo software (Flowjo, LLC). Dead cells were excluded from the analysis by setting gates in side scattering versus FL5:PB450 double scatter plots. Cell doublets were excluded by setting gates in the forward scattering area versus forward scattering height double scatter plots. The gating strategy is illustrated in Figure S21, Supporting Information. For each sample, 20 000–70 000 cells were acquired. Figures 4a–c show the average and standard deviation of the results obtained in 3 independent experiments (with the exception of the 16 h sample which was included only in two experiments).

Click-iT Plus TUNEL Assay: Cryo-sections of 4 um thickness were cut and used for TUNEL staining on the same day to detect apoptosis with the Click-iT Plus TUNEL Assay for In Situ Apoptosis Detection, Alexa Fluor 488 dye (Thermo Fisher Scientific). Sections were dried for 30 min at room temperature, fixed with 4% formaldehyde for 15 min at room temperature and permeabilised with 0.2% Triton X-100 (Sigma-Aldrich) for 15 min. Afterward, samples were washed twice in PBS for 5 min and rinsed in deionized water. Then, 100 μL of the kit's TdT reaction buffer was added for 10 min at 37 °C. Meanwhile the TdT reaction mixture was prepared according to the kit manual and 50 μL was added to the samples for 60 min at 37 °C. A humidified chamber was used to protect against evaporation. Next, the sections were washed with 3% BSA and 0.1% Triton X-100 in PBS for 5 min, rinsed in PBS and incubated with 50 μL of the Click-iT Plus TUNEL reaction cocktail for 30 min at 37 $^\circ C$ while protected from light. The sections were washed with 3% BSA in PBS for 5 min and rinsed in PBS. The samples were then incubated with CD163 antibody, as described above, or directly stained with DAPI $(1 \ \mu g \ mL^{-1})$ for 15 min at room temperature and protected from light. Finally, the slides were mounted with glass coverslips using MOWIOL 4-88 (Sigma-Aldrich). Images were acquired using a Leica SP8 confocal microscope, using a 40× objective, with a 405 nm laser for DAPI excitation, a 488 nm laser for TUNEL-Alexa Fluor 488, and a 552 nm laser for Alexa Fluor 555-CD163. Fiji-ImageJ software was used to merge multiple images of the same section as described above.

Morphological Assessment on Paraffin-Sections: Paraffin sections were stained with hematoxylin and eosin (Klinipath) (H&E) as described previously. Briefly, the paraffin sections were deparaffinized and rehydrated with decreasing strengths of ethanol (100% to 50%). Then, sections were immersed in hematoxylin (Klinipath) for 10 min. Afterward, sections were dehydrated in baths of increasing strengths of ethanol (50% to 100%) and immersed for 2 min in eosin (Klinipath). Finally, slides were mounted with glass cover slips using DePeX (Serva).

Quantification of Kupffer Cell Movement: To characterize the distance of the Kupffer cells to the slice border we used fluorescence confocal microscopy images of slices. A polygon estimating the outline of the slice was drawn and the location of all Kupffer cells within the slice was determined from the ED2 fluorescence. A few Kupffer cells close to the image border were discarded, as their closest distance to the slice border was not necessarily within the field of view. Then the closest distance from each Kupffer cells to the polygon was calculated. The distances presented in Figure 3d are pooled from several slices under the same conditions. To exclude effects due to different sizes of the imaged slices, the distances expected due to chance were estimated by Monte Carlo simulations. Thus, for each slice a position within the image was selected at random. If the position did not fall within the polygon outlining the slice, then it was rejected; otherwise, its distance to the border was calculated. This was repeated for 100 000 positions per image to allow a good estimate of the distances expected due to chance. The proportion of Kupffer cells within 20 μ m quantified experimentally was then normalized (divided by) the proportion estimated to be due to chance. Number of slices/number of Kupffer cells per condition: 24 h control, 5 slices/305 Kupffer cells; 48 h control, 2/219; 72 h control, 6/781; 24 h treated, 5/283; 48 h treated, 2/208; 72 h treated, 6/790.

Statistical Analysis: Normal distributions could not be assumed due to small sample size ($n \le 8$). Therefore, statistical differences between two groups were assessed using nonparametric Mann-Whitney U for unpaired data or Wilcoxon for paired data. For the comparison of multiple groups, Kruskal Wallis was used for unpaired data or Friedman for paired data. p < 0.05 was considered significant. The data were SCIENCE NEWS _____ www.advancedsciencenews.com

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analyzed using GraphPad Prism 8, except for Figure 3e where scipy version 0.19.1 was used.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

R.B. designed and performed all experiments, analyzed and interpreted the data, and drafted the manuscript. C.Å. performed the quantification of Kupffer cell distribution and revised the manuscript. B.N.M. contributed to the optimization of tissue digestion and flow cytometry sample preparation and data analysis, and revised the manuscript. Y.L.B. and P.O. designed the experiments, interpreted the data, and revised the manuscript. A.S. designed the experiments, interpreted the data, and wrote the manuscript.

Keywords

advanced models, ex vivo models, Kupffer cells, liver, nanoparticle interactions, nanosafety, tissue slices

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