Mobile Magnetic Nanocatalysts for Bioorthogonal Targeted Cancer Therapy

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The use of magnetic nanorobots to activate chemotherapeutic prodrugs represents a promising alternative to current chemotherapeutic treatments. Here, a hybrid nanowire (NW) for targeted bioorthogonally driven activation of the latent chemotherapeutic prodrug 5-fluoro-1-propargyl-uracil (Pro-5-FU) in vitro and in vivo cancer models is proposed. The NWs are composed of magnetic iron (Fe) and palladium (Pd), a known bioorthogonal catalyst. In vitro tests with a cancer cell line showed no significant cytotoxic effect by the NWs. In contrast, NWs combined with Pro-5-FU lead to a significant reduction of cell viability, similarly to the one induced by its active chemotherapeutic counterpart 5-fluorouracil (5-FU). The reduction in cell viability is attributed to the catalytic activation of Pro-5-FU into 5-FU. To demonstrate their targeted therapeutic abilities, magnetic fields are used to attract the FePd NWs to a predefined area within a cultured cancer cell population, causing a local Pro-5-FU activation, and subsequent cell death in this region. As a proof of concept, NWs are injected in cancer tumor xenografts. The intraperitoneal injection of Pro-5-FU significantly retards tumour growth without causing significant side effects. This work presents a novel chemotherapeutic approach combining nanorobotics and bioorthogonal activation of prodrugs as an efficient alternative to conventional chemotherapy.

1. Introduction

Tremendous research in the field of cancer therapy has opened up new avenues for treating this devastating disease.[1] In general, the main goal of cancer treatment is to deliver high doses of antitumor agents to the site of action, while minimizing side effects within the healthy surrounding tissue.[2] A majority of current clinical chemotherapy approaches distribute chemotherapeutic agents throughout the entire body. These treatments lack target specificity and are primarily limited by the reduced target drug concentration and nontargeted tissue distribution.[3] These shortcomings render current treatment methods prone to cause fatal side effects for the patients.[4] Further limitations are the fast deactivation, unfavorable pharmacokinetics, and early clearance of the anticancer drug from the body.[5,6] A promising path to overcome these problems can be obtained from the progress made...
in the field of nanobiotechnology, by tailoring advanced nanomaterials to allow for site-specific targeting and on-demand drug activation.[7]

One approach to restore the clinical use of promising anticancer drugs, with limited pharmacokinetic profiles, is to transform chemotherapeutic agents into latent prodrugs, which are only medically effective upon catalytic activation.[8] This strategy has led in the past to clinical trials and approvals of conventionally unfavorable anticancer drugs.[9–11] Initially, the activation of biolabile prodrug systems was dependent on a metabolic activation via enzymes in the organism. However, this approach requires either a change in the metabolic environment of the host by artificially introducing enzymes in the body, or lacks target specificity due to the nonspecific abundance of this enzyme throughout the whole organism.[12] A second class of prodrugs, so called the bioorthogonal prodrugs, are inspired by the work of Bertozzi on copper free azide–alkyne cycloaddition and Staudinger ligation.[13] Bioorthogonal prodrugs are physiologically stable precursors, which can be activated through biocompatible heterogeneous catalysts by means of bioorthogonal organometallic (BOOM) reactions.[14] Novel strategies are currently focused on transition metals as catalysts for BOOM reactions, such as copper (I), ruthenium (II), and rhodium (II). However, only a few known reactions are suitable to work under physiological conditions without causing toxic side effects.[15–17]

Palladium (Pd), being biocompatible[18–20] and exhibiting strong catalytic activity, is a promising transition metal candidate for bioorthogonal reactions.[5,9] Recent research by Weiss et al. demonstrated the spatially defined Pd mediated bioorthogonal activation of 5-fluoro-1-propargyluracil (Pro-5-FU) and N-propargylxycarbonylgemcitabine prodrugs to their respective anticancer agents 5-fluorouracil (5-FU) and gemcitabine.[22,23] Although the prodrugs were biologically inert, their activation by Pd particles embedded in a matrix of glycol–polystyrene resin was sufficient to deprotect the respective propargyl group and restore cytotoxicity and antiproliferative properties. In this context, intratumoral implants loaded with Pd nanoparticles have been successfully used as bioorthogonal catalysts in in vitro cancer models and in vivo zebrafish. However, further in vivo demonstrations, particularly in mammalian model systems, are crucial to assess the actual therapeutic potential with regards to safety and pharmacological profiles. Furthermore, it is desirable to explore other means of device design to administer the transition metal Pd, directly to the tumor site, to render it minimally invasive.

The use of nanorobots for targeted drug delivery has tremendously impacted the field of medical research within the last decades.[24,25] Nanorobots have potential benefits over current state-of-the-art medical procedures, such as: (i) targeted drug delivery with increased bioavailability; (ii) reduced human error through computer controlled automated delivery processes; (iii) minimally invasive remote access inside the body; (iv) minimization of undesired side effects; (v) early diagnosis; and (vi) constant monitoring.[26,27] Recently, the scientific focus has been on the development of smart micro and nanorobots to transport and deliver pharmaceutical agents to the respective target site. Various manipulation techniques (light, electromagnetic fields, acoustic waves) together with chemical or biological targeting agents further enhanced the target specificity of such devices in vitro and in vivo.[28–32] The main challenges of these small-scale robots are still related to the transport of pharmacologically sufficient concentrations of the drug to the target site and the implementation of an efficient remote control release mechanism, exclusively at the region of interest.[33]

In this work, we report for the first time, a FePd nanorobot that is capable to target specific sites in the cancer cell population and perform bioorthogonal organometallic activation reactions of the commercially available prodrug Pro-5-FU in vitro. We applied template-assisted electrodeposition for the batch fabrication of heterogeneous FePd nanowires and demonstrated their capability for bioorthogonal activation of Pro-5-FU in an in vitro breast cancer model—the MDA-MB-231 cell line. Using the ferromagnetic properties of Fe within the structure, we successfully showed precise, wireless locomotion and applied this property to demonstrate spatially targeted activation of Pro-5 FU in vitro. Additionally, we applied the magnetic and bioorthogonal active nanorobots to an in vivo mouse model. We were able to demonstrate for the first time, the therapeutic potential of nanorobots for Pd mediated BOOM reactions of Pro-5-FU, with respect to therapeutic efficiency and safety, by significantly reducing MDA-MB-231 tumor growth in nude mice. Combining nanorobots with bioorthogonal chemistry represents a novel avenue in cancer therapy, potentially minimizing the side effects of conventional chemotherapeutic anticancer drugs and retaining high pharmacological efficiency in the tumor region.

2. Results and Discussion

The use of nanorobots in biomedical applications demands an assembly strategy that allows for reliable and controlled batch-fabrication of the device. The FePd nanorobots in this work were fabricated by template-assisted electrodeposition in anodized aluminum oxide (AAO) templates (Figure 1a). Coelectrodeposition of Fe (II) and Pd (II) is a challenging system due to the large differences in standard potential (Fe (II) – +0.44 V; Pd – +0.98 V). An electrolyte solution composed of only the two metals initiates a redox reaction, causing oxidation of Fe(II) to Fe(III) and precipitation of reduced Pd(II) as well as Fe(OH)₃

\[
Pd^{2+} + 2Fe^{2+} \rightarrow Pd^{0} + 2Fe^{3+} \quad (1)
\]

Therefore, the addition of complexing agents is needed in order to bring the standard potentials of both metals closer together and to avoid further redox reactions and precipitation of the constituents in the electrolyte. It has been reported that ammonia sulfate and ammonia citrate are effective stabilizing agents, which prevent ferric hydroxide precipitation and further reduce the noble standard potential of Pd according to the following reactions:[34,35]

\[
PdCl₂ + 4NH₃ \rightarrow [Pd(NH₃)₄]Cl₂ \quad E^0 = 0.0 \text{ V} \quad (2)
\]

\[
Fe^{3+} + 5NH₃ + 2C₂H₃O₂⁻ \rightarrow (NH₄)₂[Fe(C₂H₃O₂)₂]⁺ + H₂ \quad E^0 = -0.014 \text{ V} \quad (3)
\]
Subsequently, the electrolyte bath was prepared by forming this tetraamine palladium (II) chloride complex by adding ammonia sulfate at a pH of 1 until all of the Pd was dissolved. Afterward, ammonium citrate dibasic was added to allow the introduction of FeCl₃ salts. Next, after vigorous stirring for 2 h, we received a clear solution and used ammonium hydroxide to increase the pH value to 9. The resulting electrolyte bath (Table S1, Supporting Information) was used for electrodeposition of FePd nanowires at a current density of \(-2\) mA cm\(^{-2}\).

Scanning electron microscopy image of the resulting FePd nanowires with an average length of 4 \(\mu\)m is shown in Figure 1b. Transmission electron microscopy (TEM) electron diffraction analysis in Figure 1c confirmed the presence of a polycrystalline alloy of FePd. Interestingly, electrodeposited FePd nanowires (Figure 1d,e) showed a homogeneous distribution of Fe and Pd across the surface. Here, TEM-energy dispersive X-ray spectroscopy (EDX) analysis revealed the distribution of Fe (=50 at\%) and Pd throughout the nanowire. Annealing the nanowires at 700 °C induced the segregation of both elements, leading to the formation of Fe- and Pd-rich regions across the nanowire (Figure 1f,g).

Next, we assessed the biomedical applicability of our fabricated FePd nanowires as a catalyst for the bioorthogonal prodrug activation in vitro. For the purpose of this work, we have chosen MDA-MB-231 breast cancer cell line, since breast cancer is a highly heterogeneous disease, which represents the most commonly diagnosed and second leading cause of death in women in the western hemisphere.[36] MDA-MB-231 cell line was derived from a metastatic pleural effusion of a breast cancer patient and has been extensively studied for in vitro and in vivo experiments. A scheme of action of the proposed system is represented in Figure 2a. Upon application of the biochemically inert prodrug Pro-5-FU, no effect on an in vitro breast cancer model (MDA-MB-231) is observed. However, the introduction of the FePd nanorobots is expected to catalyze the bioorthogonal deprotection of Pro-5 FU by cleaving off the attached propargyl group. The resulting biochemically active 5-FU anticancer agent will then enter the cancer cell, followed by its conversion to the active metabolite by deoxyuridine monophosphate induced apoptosis. In the following experiment, the colorimetric MTT assay was used for the quantification of metabolic activity that corresponds to cell viability.

Pd is already an established and proven biocompatible material frequently used in implantable medical devices.[37] However, the biocompatibility of electrodeposited Fe nanowires is somewhat controversial.[38,39] Hence, before testing the functionality of our FePd nanowires, it was pivotal to determine the biocompatibility of the electrodeposited FePd nanorobots toward cancer and noncancerous cell lines. Figure 2b presents the results of the colorimetric MTT assay of MDA-MB-231 cells conducted with increasing concentrations of FePd nanowires even up to 200 ppm. The results show no significant reduction of cell viability toward increasing FePd concentrations. This effect was also found on human 3T3 fibroblast cells cocultured with 100 ppm of FePd nanowires. Even after 72 h no apparent morphological changes have been observed at increased nanowire concentrations (Figure S1a,b, Supporting Information), rather 3T3 cells started to interact with the nanowires by either internalization or growing on top of them (Figure S2, Supporting Information). Also, the corresponding cell viability assay did not show any cytotoxic effects up to 200 ppm of FePd (Figure S1c, Supporting Information).

Next, we tested the biochemical activity and inertness of 5-FU and Pro-5-FU, respectively. Three different concentrations of the drug and prodrug were added to MDA-MB-231 breast cancer cells and cell viability was assessed after 72 h by MTT (Figure 2c). As expected, 5-FU significantly reduced the cell viability by 50% at \(0.2 \times 10^{-3}\) m and 70% at \(1 \times 10^{-3}\) m, when compared to cells treated with the biochemical inert Pro-5-FU (5–20% at 0.2 or \(1.0 \times 10^{-3}\) m).
After confirming the biocompatible properties of the FePd nanowires, as well as the biochemical inertness of the Pro-5-FU, we went one step further to assess the potential of FePd as a bioorthogonal catalyst under physiological conditions in a MDA-MB-231 cell culture. In this case, breast cancer cells were cultured with Pro-5-FU (0.5 × 10^{-3} M). After cell attachment, various concentrations of annealed and as electrodeposited (not annealed) FePd nanowires were introduced to the cell culture. Figure 2d shows that an increasing amount of FePd nanowires (annealed/not annealed) led to a significant reduction in cell viability compared to the control experiments. The effective conversion efficiency of 100 ppm FePd nanowires was quantified by high-performance liquid chromatography measurements to be at 67% after 72 h of incubation (Figure S3, Supporting Information). In fact, these results proof for the first time that FePd-based nanorobots are capable of inducing the bioorthogonal conversion of Pro-5-FU to 5-FU.

Next, we tested the efficiency of our system based upon varying amounts of Pro-5-FU at a constant FePd nanowire concentration (100 ppm). Figure 2e shows the MTT results of four different Pro-5-FU concentrations in combination with annealed or not annealed nanowires. Again, the bioorthogonal activation of Pro-5-FU resulted in a significant reduction in cell viability, even at the lowest concentration of 0.5 × 10^{-3} M. Increasing Pro-5-FU concentrations further reduced the MDA-MB-231 cell viability. Interestingly, upon a concentration of 1.5 × 10^{-3} M Pro-5-FU, the annealed FePd nanowires showed a significantly increased efficiency of the Pro-5-FU compared to the as-electrodeposited FePd samples. Considering the redistribution upon annealing of the Fe and Pd on the nanowire (as shown in Figure 1g), we assume that the formation of Pd-rich regions increased the local Pd concentration on the nanowire surface. Consequently, the higher amount of Pd on the surface is expected to increase the conversion efficiency of Pro-5-FU and hence, lead to an augmented pharmacological profile.
After we have confirmed the bioorthogonal capability of FePd nanowires, we tested the application of our system for spatially targeted cancer therapy. Due to the ferromagnetic properties of Fe in the nanowires, wireless magnetic manipulation can be applied for a noninvasive and precise locomotion of the nanorobots to the target site. In order to simulate such a setup, MDA-MB-231 cells were cultured in a Petri-dish and incubated with Pro-5-FU and 100 ppm FePd nanowires (Figure 3i). Afterward, we placed a magnet under a defined area of the tissue culture dish. After 72 h, we used a LIVE/DEAD staining kit for the cells and imaged different parts of the entire culture. The two fluorescent dyes of the kit, SYTO9 and propidium iodide, allowed differentiating the alive (green) and dead/compromised cells (red), respectively. On the left site (ii) (region without the magnet), we observed significantly lower amounts of FePd nanowires and most of the cells present in that area showed a green (alive) fluorescence signal. On the other hand, the right site (iii) was heavily populated with FePd nanowires due to the attraction by the applied magnet. Consequently, in that area FePd nanowires could effectively catalyze the bioorthogonal conversion of Pro-5-FU to 5-FU and hence cells in that region were predominantly compromised, as indicated by the red fluorescence signal. Furthermore, we have verified the mobility of FePd nanowires using uniform low-magnitude rotating magnetic fields. Video S1 (Supporting Information) demonstrates the guided locomotion of the FePd nanowires along a square trajectory.

Finally, we validated the in vitro results in an in vivo preclinical mouse model. For this, breast cancer cells (MDA-MB-231) were subcutaneously injected in the right flanks of female nude mice (Figure 4a). Tumors were allowed to grow for a week, until reaching a mean average tumor volume of 100 mm³. At this time, mice were randomly distributed in five groups; two of them (group 4 and group 5) received an intratumoral injection of a FePd nanowire suspension (25 mg kg⁻¹) in 100 µL phosphate buffered saline (PBS), while the other groups (Groups 1, 2, and 3) were injected with the same volume of PBS. Thereafter, three times a week for a total period of two weeks, mice were intraperitoneally injected with active 5-FU (40 mg kg⁻¹; Group 3) and inactive Pro-5-FU (40 mg kg⁻¹, Groups 2 and 5) (Figure S4, Supporting Information). Groups 1 and 4 received the vehicle alone. Figure 4b shows the development of relative tumor volume growth of all five groups. Interestingly, the groups treated with the active drug 5-FU (Group 3) and FePd nanowires in combination with Pro-5-FU (Group 5) showed a significant reduction in tumor growth, when compared to the control mice (PBS only (Group 1); Pro-5-FU only (Group 2); FePd nanowires only (Group 4)). Moreover, tumor growth inhibition of the activated prodrug was comparable to the active chemotherapeutic agent. This result confirms the in vivo activation
of the Pro-5-FU by FePd nanowires injected in the tumor. The administered nanowires were observed throughout the experiment in the tumor region of all mice (Figure 4c), which was further confirmed after dissection of the tumor (Figure 4d). Further, histological evaluation of the recovered tumors from mice showed a decrease in vascular invasion in mice treated
with FePd nanowires and Pro-5-FU, as well as with only 5-FU (only 1 in 4 mice showed signs of vascular invasion). This result suggests that the biological effect promoted by the active 5-FU and FePd activated Pro-5-FU are comparable (Figure 4e). Additionally, biochemical analysis of the blood for liver (Figures S3a and S6, Supporting Information) and kidney (Figure S5b,c, Supporting Information) toxicity markers, such as alanine aminotransferase, urea, and creatinin, respectively, did not show any significant differences between FePd nanowires, Pro-5-FU, and 5-FU groups. The fact that 5-FU did not show any indications of side effects could mainly be attributed to the short time frame of the experiment, since previous studies reported only significant long-term side effects in vivo. The results were also validated histologically, where no differences between the liver and kidney tissue of the animal groups was observed (Figures S6 and S7, Supporting Information). Furthermore, immunohistochemical staining for ki67 (a cellular proliferation marker), showed the same trend as for tumor growth and for vascular invasion, where both groups 5-FU and FePd nanowires with Pro-5-FU, have a decrease in the percentage of positive (proliferating) cells (91.25+/−1.25 vs 81+/−3.317 (Pro-5-FU vs 5-FU) (p = 0.0346) and 87+/−3 vs 83+/−1.225 (FePd nanowires with PBS vs FePd nanowires with Pro-5-FU) (Figure S8, Supporting Information). The FePd nanowires were identified both in the histological sections (Figure S7, Supporting Information) and by TEM analysis (Figure 4f–h). Through TEM analysis, it was possible to confirm that the FePd nanowires were uptaken by the breast cancer cells, but had no influence on their morphology or cell viability (Figure 4f,g). Apart from this, in mice treated with FePd nanowires and Pro-5-FU, the TEM observation again confirmed the cellular uptake of nanowires and further showed a significant increase in the number of dead and apoptotic cells within the tissue (Figure 4h).

3. Conclusion

In this work, we combined for the first time a magnetically driven, nontoxic nanorobot for the bioorthogonal activation of the chemotherapeutic prodrug—Pro-5-FU—and tested the effect of this system for cancer therapy. The nanowires composed of Fe and Pd entities were synthesized by template-assisted electrodeposition. Biocompatibility tests, both in vitro and in vivo, did not show any cytotoxic effects, enabling the use of FePd nanowires for biomedical applications. Next, we demonstrated the bioorthogonal conversion of the latent Pro-5-FU to the active chemotherapeutic 5-FU in the presence of FePd nanowires by initiating significant cell death in a breast cancer in vitro cell model. The magnetic properties of Fe present in the nanowires allowed us to extend our in vitro study to demonstrate targeted cancer therapy. Here, we used magnetic fields to attract FePd nanowires to predefined cancer areas, triggering the local activation of Pro-5-FU and subsequent cell death exclusively in this region. Finally, we transferred the findings of our novel approach to an in vivo mice model of MDA-MB-231 human breast cancer xenografts in nude mice. The FePd nanowires injected in the tumor significantly reduced tumor growth for intraperitoneal administration of Pro-5-FU over a period of two weeks compared to the control groups (only PBS, only Pro-5-FU, only FePd).

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.

Keywords

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