Noninvasive in vitro and in vivo Monitoring of Drug Delivery Processes by Electron Spin Resonance, Benchtop-NMR and Multispectral Optical Imaging

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ABSTRACT SUMMARY
The performance of a drug delivery system in vitro and in vivo is a result of complex interactions between the drug, the carrier and the release environment. The rationale design and improvement requires appropriate analytical methods. It will be illustrated how electron spin resonance (ESR, EPR), benchtop-NMR and multispectral optical Imaging can be used to monitor drug delivery processes noninvasively in vitro and in vivo. Examples range from in situ implants to stimulus responsive drug release from polymer conjugates. Unique information on the microclimate (viscosity, pH, polarity) can be obtained.

INTRODUCTION
Many concepts have been developed to improve drug delivery systems. The careful tuning of the composition, the material properties, size, charges, flexibility and other parameters is commonly applied to adapt the release profile to the medical need. In addition, the introduction of stimulus responsive characteristics offers new avenues to improve efficiency and to decrease side effects. However, promising data obtained in vitro are often followed by discouraging in vivo results. One of the main reasons is the fact, that very often the complex in vivo situation is poorly reflected by in vitro tests.

For example, protein adsorption, phagocytosis, enzymatic degradation or encapsulation might change the fate of drug carriers significantly from the behavior in PBS buffer.

It is therefore highly desirable to follow drug delivery processes in vitro and in vivo. Appropriate analytical methods should be sensitive and specific to key processes of drug delivery. The characterization is highly challenging due to the complex composition of a living organism. Although the monitoring of implants and microparticles might already been challenging, the characterization of nanosized drug delivery systems is even more difficult. The presented data show exemplary, how ESR, BT-NMR and multispectral optical Imaging can be used to characterize drug delivery processes in vitro and in vivo.

EXPERIMENTAL METHODS
The experimental details are published in the references.

RESULTS AND DISCUSSION
Multispectral Optical Imaging permits the recording of spatially resolved emission spectra. By means of the spectral database it is possible to resolve the spatial distribution of several species. This method is very useful in preclinical drug delivery and pharmacotherapy studies. For example, it is possible to monitor tumor growth and the response to chemotherapy in fluorescent xenografts quantitatively [1]. In addition, it is possible to follow the fate of stimulus sensitive polymer therapeutics as illustrated in Fig. 1.

Figure 1: Top: Principle of pH-sensitive delivery from polymer conjugates. Bottom: In
vivo distribution of the polymer backbone (dye A) and the pH-cleavable dye (dye B).

Both dyes showed comparable distributions at early time points. With time, a higher accumulation of the pH-cleavable dye in the tumor was found [2]. The strong size dependency of blood circulation and tumor accumulation for pegylated nanoparticles could also be shown [3,4]. Another study proves the excretion of 195 kD PVA and its association with fat tissue which caused a spectral shift. It was therefore possible to monitor the distribution and kinetics of interacting and not interacting PVA simultaneously [5].

Electron Spin Resonance (ESR) is a very valuable tool in preclinical drug delivery research [6]. Due to the dielectric loss, lower frequencies (1 GHz) are required to monitor water containing pellets, tablets or small mammals. By means of spin probes it is possible to measure important parameters of drug delivery such as microviscosity, microacidity and micropolarity. In addition, special materials permit also measurement of the local oxygen concentration. By means of ESR it could be shown, that the pH inside degrading PLGA might reach very low values (pH 2) [7]. Recent applications include the detection of pH-gradients in tablets [8] and the quantitative in vitro and in vivo monitoring of solvent exchange and precipitation processes of in situ implants [9, 10].

Nuclear magnetic resonance based methods (NMR, MRI) offer a large variety of different opportunities in the field of drug delivery. Proton NMR can be used to quantify the changing film composition of coated dosage forms [11]. The development of low cost benchtop instruments (BT-NMR) should stimulate a broader use of this method. BT-NMR relaxation studies can be used to monitor gelation processes or to study solid-liquid interactions in adsorbates [12] or selfemulsifying pellets. During the last years, BT-MRI has been developed. Despite the lower resolution of these systems compared to high-field MRI machines, useful applications are possible. Examples include the monitoring of in situ implants [10] and the tumor growth in mice [13]. Human studies often require a magnetic labeling, but single pellets can be detected [14].

CONCLUSION
Multispectral Optical Imaging, ESR and NMR are noninvasive methods which permit the noninvasive monitoring of drug delivery processes in vitro and in vivo. Unique and valuable information can be obtained which facilitates a rational based development of drug delivery systems.

REFERENCES

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