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Monitoring drug release and polymer erosion from therapeutically used biodegradable drug carriers by EPR and MRI *in vitro* and *in vivo*

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The in vivo performance of anticancer drugs is very often limited by their poor bioavailability, serious side effects and short half lives. During the last years, biodegradable drug carriers have been developed to overcome these problems. Despite the clinical use of these delivery systems, there is a rather poor understanding of the detailed mechanisms of drug release and polymer erosion, particularly in vivo. The paper demonstrates how noninvasive magnetic resonance techniques ESR and MRI may contribute to increase our understanding of the in vivo fate of biodegradable drug delivery systems.

Key words: antineoplastic agents; infusion pumps, implantable; drug carriers; biodegradation; magnetic resonance imaging; electron spin resonance spectroscopy

Introduction

The therapeutical use of drugs is often limited by their poor bioavailability, short half lives and serious side effects. To overcome these problems, biodegradable drug delivery systems (BDDS) have been developed which provide a local release at a desired rate (from days to months). Several biodegradable drug carriers containing GnrH agonists are now commercial products for the treatment of prostate cancer. Examples include biodegradable implants (PROFACT-DEPOT®, ZOLADEX®) and microparticles (DECAPEPTYL-DEPOT®, ENANTONE-DEPOT®). They are implanted or injected subcutaneously and provide a controlled drug delivery from 1 to 3 months. The matrix of these systems is made from poly(α-hydroxy-esters) and degrades *in vivo* into lactic and glycolic acid. Another example of the clinical use of BDDS is the treatment of glioma with the GLIADEL™-implant. In this case, BCNU is released locally over 2-3 weeks from polyanhydride wafers implanted in brain. Compared to intravenous injection, this approach results in higher local, but lower systemic drug concentrations, thereby enhancing the therapeutic effect and reducing serious side effects of the drug.

The *in vivo* performance of the BDDS results from the complex interaction between the drug, the polymer and the biological sys-

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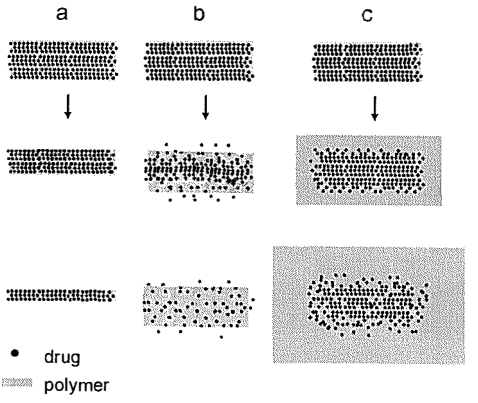

Figure 1. Principle schemes of erosion- (a), diffusion- (b) and swelling- (c) controlled drug release.

tem. Despite the clinical use and the encouraging results which have been obtained, there is still a rather poor understanding of the detailed mechanisms of drug release and polymer degradation. For example, the causes for different results observed *in vitro* and *in vivo* remained speculative.[1]

In general, the incorporated drug may be released by three different mechanisms (Figure 1). The mechanism that actually determines the overall release kinetics results from the ratio of the kinetics of water penetration, drug solubilization and diffusion, polymer swelling and polymer erosion. For example, in the case of erosion controlled release, the rate of polymer erosion is faster compared to the rate of drug diffusion and polymer swelling. As long as a constant surface area exist, a constant drug release can be realized by erosion controlled systems (Figure 1 and Table 1). In the case of diffusion and swelling controlled drug release, the release rate decreases with time due to the increase in the distance to the polymer surface. It is important to realize that the exposure of the solubilized drug to the polymer microenvironement prior to the release may cause drug hydrolysis and drug inactivation. These processes are more likely to occur in diffusion and swelling controlled drug release.

An appropriate characterization *in vivo* is a prerequisite to achieve progress in the improvement of the existing systems and the development of new BDDS with optimized profiles of drug release and polymer clearance. The desired characteristics of the analytical techniques include sensitivity for key processes of drug release and polymer erosion (water penetration, solubilization of the drug, polymer degradation and erosion) and

Table 1. Characteristics of different drug release mechanisms

	Drug release mechanism		
	Erosion controlled	Diffusion controlled (case I)	Swelling controlled
Release kinetics for tablet shape (diameter >> thickness)	Constant release (zero order)	release rate decreases with time ($\sim \sqrt{t}$)	release rate decreases with time ($> \sqrt{t}$)
Polymer erosion (mass loss)	simultaneous with drug release	after drug release	after drug release
Size of the polymer matrix	decreases simultaneously with drug release	remains constant during time of drug release	increases during time of drug release
Time of drug solubilization inside the matrix prior to release	zero of very short	long	long
Danger of drug decomposition prior to release	low	high	high

noninvasiveness. The drawbacks of the analytical methods currently employed (size exclusion chromatography, differential scanning calorimetry, electron microscopy) necessitate sample separation from the biological surrounding which is difficult for micro- and nanoparticulate systems and may lead to artifacts. Isotopic labelling does not permit the characterization of key processes of drug delivery, such as water penetration and polymer degradation. Magnetic resonance based techniques are promising candidates to follow BDDS *in vivo* due to their noninvasiveness and their sensitivity to water concentration and water mobility. The development of low frequency EPR spectrometers makes it now feasible to conduct noninvasive measurements on living mammals.[2] The sensitivity is high enough to detect free radicals derived from xenobiotics[3] or drugs[4] and reactive intermediates of metal ions.[5,6] Spin trapping techniques can be used to detect and image radicals with short half lives.[7,8,9] The following examples illustrate how electron paramagnetic resonance spectroscopy (EPR) and nuclear magnetic resonance imaging (MRI) give unique information about the processes of drug delivery and polymer degradation *in vitro* and *in vivo*.

Results and discussion

Gamma irradiation is widely used to sterilize BDDS. Some drug or polymer derived radicals which are formed during irradiation are very stable at room temperature under dry conditions. However, they will decay immediately after water induced solubility of the surrounding matrix. Therefore, these endogenous signals may be used to compare the velocity of water penetration between *in vitro* and *in vivo*. The realization of this concept has been demonstrated on gentamicin loaded polyanhydrides, which were subcutaneously implanted in mice.[10]

The application of EPR can be expanded to diamagnetic BDDS by the introduction of nitroxyl radicals. Low molecular weight nitroxides may serve as model drugs. Another possibility is to use spin labelled drugs (for example spin labeled peptides) or spin labelled polymers. A large variety of nitroxides permits the choice of the compound with the most appropriate characteristics (mol. weight, hydrophilicity, acidity etc.). The EPR spectra give information about:

1. nitroxide concentration (by double integration of the EPR spectra)
2. micropolarity (by the hyperfine coupling constants)
3. microviscosity (by the shape of the EPR spectra)

which can be used to elucidate the release mechanism. Figure 2 demonstrates how the percentage of water solubilized and nonsolubilized nitroxides can be estimated by spectral simulation. The percentage of undissolved nitroxides is easily underestimated in the EPR spectrum due to the large line width which leads to small signal amplitudes. Therefore, the integration of spectra is desirable. It is possible to indicate on the mechanism of drug release directly from the information of the EPR spectra. Results of previous studies demonstrate that diffusion controlled processes contribute to the release mechanism of clinically used polymers.[11,12,13] Erosion controlled release was observed only in the case of the polyanhydride biscarboxyphenoxypropane, a polymer which is used clinically to deliver BCNU against glioma.[13] The EPR method is also able to follow complexe release mechanisms: Drug release from poly(fatty acid dimer-sebacic acid) polymers involves water penetration, polymer degradation with precipitation of the monomers and incorporated drug molecules, resolubility and diffusion.[12] Further information on the release processes can be

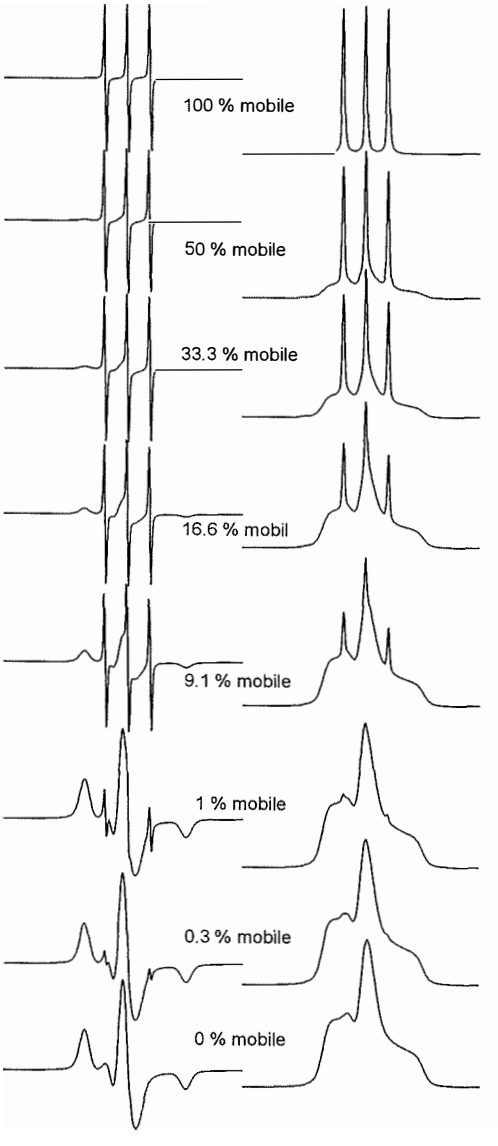

Figure 2. Spectral simulation of the EPR spectra (1. derivative of microwave absorption, left) and their integrated form (right) of the superposition of water solubilized, mobile nitroxides and non solubilized nitroxide molecules. Note that the signal amplitude of the water solubilized nitroxides is much higher than the signal amplitudes of the nonsolubilized form due to the narrow line width.

achieved by simultaneous monitoring of distinct polymer layers which can be realized *in vitro* by spectral-spatial EPR-imaging.[14] The spatial resolution of the current *in vivo* EPR imaging machines is in the range of few millimeters [15] and not sufficient to resolve heterogeneity within a millimeter sized implant. However, a distinct regions of the implant can be separated by means of different nitroxide isotopes.[13]

An important parameter is the pH inside the degrading polymer matrix. The acidity influences the polymer degradation rate, solubility of the incorporated drug and drug stability. An acidic pH may result from the polymer degradation, because the polymers which are clinically used degrade into α-hydroxyacids (polyesters) or dicarboxylic

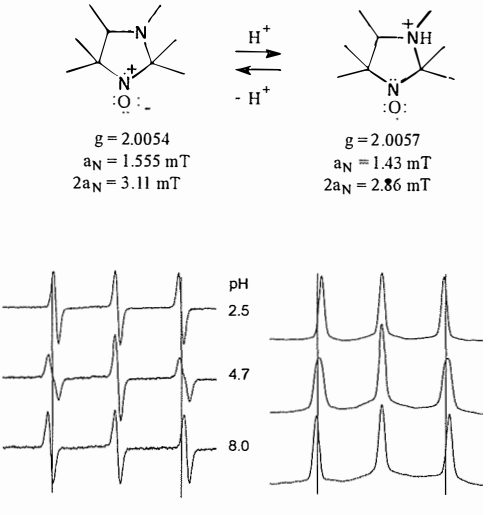

Figure 3. *Top:* Basic principle of the pH-measurement by imidazolidine nitroxides. Protonation of the nitrogen in position 3 decreases the spin density of the nitrogen atom of the radical moiety, which results in a decreased hyperfine splitting constant and an increased g-value. *Bottom:* Influence on the pH on the experimental 1.1 GHz EPR-spectra (left) and their integrated form of the pH-sensitive nitroxide 2,2,3,4,5,5-Hexamthyl-imidazolidine-1-oxid. The EPR spectrum at pH = pKa = 4.7 results from a superposition of the protonated and the nonprotonated form of the radical.

acids (polyanhydrides). However, the acidity inside the matrix is difficult to predict due to an uncertain monomer concentration and possible influences of incorporated drugs or penetrating ions. There was no information available about the acidity of the microenvironment inside BDDS *in vivo* due to the lack of suitable techniques. The development of pH-sensitive nitroxides [16] made it possible to study the pH inside degrading polymers non-invasively and continuously *in vivo* (Figure 3) A pH drop from 5 to 2 was observed in biodegradable polyester implants in mice.[11] Clearly, such an acidic microenvironment may lead to drug decomposition prior to drug release.

Complementary information can be obtained by the combination of the EPR studies with nuclear magnetic resonance imaging.[12,13] MRI provides information concerning the implant shape and size, edema and encapsulation. The cause of incomplete nitroxide release was found by the MRI detection of the encapsulation of the implant.[12] Care must be taken to conclude from low MRI contrast to the absence of water inside the implant, because small pore sizes may lead to very short relaxation times. Therefore, drug release may be completed, although no increase in MRI signal intensity was observed.[12,13] This water can be detected indirectly by EPR using spin probes.

In summary, drug release from BDDS is a promising approach in the field of cancer treatment. Magnetic resonance techniques EPR and MRI can provide unique and additional information needed to understand the mechanisms of drug release and polymer degradation. They permit also the *in vivo* characterization of submicron sized delivery systems,[17] which are otherwise only detected by radiolabeling. Ongoing developments in the field of EPR-imaging will result in new opportunities to monitor the localization and physical state of the delivery system (hydration, microviscosity, micro-pH).

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