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Noninvasive in vivo EPR monitoring of the methyl methacrylate polymerization during the bone cement formation

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Abstract

The curing of poly(methyl methacrylate) (PMMA) bone cement is done by a free radical polymerization. As the amount of free radicals present is a marker of the amount of unpolymerized chains present in the polymer, it is assumed that this could be related to the mechanical properties such as strength or density. In this study, the direct observation of the free radicals produced during the PMMA bone cement formation was obtained for the first time in vivo using low-frequency EPR spectrometers (1.2 GHz). Low frequency permits measurements in live animals due to the increased microwave penetration. The amount of polymerization radicals was carried out noninvasively over days on the same animals. The decay rates obtained in vitro and in vivo were compared: the decay rates were significantly lower when the curing process occurred in vivo compared to the situation in vitro. As the kinetics are rather different in vitro and in vivo, this emphasizes the value of the present method that permits the noninvasive monitoring of the curing process directly in vivo.

Keywords: EPR; ESR; Vivo; Bone cements; Poly(methyl methacrylate)

1. Introduction

The use of auto-polymerizing poly(methyl methacrylate) (PMMA) based bone cements is the most common method to secure prostheses. Intense research still is conducted to improve the biocompatibility and the solidity of these materials. Most studies have concentrated on the macroscopic properties of the cement [1–4]. PMMA cements are usually composed with preformed PMMA beads being mixed with methyl methacrylate monomer. The polymerization process occurs as a result of the reaction between the benzoyl peroxide in the polymer powder and *N*,*N*-dimethyl-*p*-toluidine in the monomer, and the acrylics can be polymerized by a free radical chain reaction process. The group of Park demonstrated the feasibility of monitoring the molecular processes occurring in PMMA bone cement formation by using electron paramagnetic resonance (EPR) spectroscopy to observe the growing monomer free radicals (Fig. 1) [5]. The generation and the decay of free radicals were compared to the mechanical properties [6]. The influence of the initial temperature, the influence of mixing ratio, the effect of accelerators of polymerization were studied by EPR [7–9]. As the amount of free radicals present is an indication of the amount of unpolymerized chains present in the polymer, it is assumed that this could be related to mechanical properties such as strength or density. In the few studies that compare bone cements samples cured in vitro with samples that were implanted in vivo, it was clear that the kinetics of decay of free radicals were different in both situations. It is clearly debatable to give a predictive value to experiments where the curing process is monitored in vitro and to extrapolate this kinetics to the situation in vivo. It should be more convenient to look on samples aged in vivo. Up to now, it was only possible to evaluate the radical process ex vivo (samples removed from the body) using the conventional 9 GHz EPR spectrometers. Recent progress in EPR spectroscopy techniques now permits sensitive measurements in living animals. The use of low-frequency EPR spectrometers permits an increased wave penetration into tissues and has led to an increasing number of

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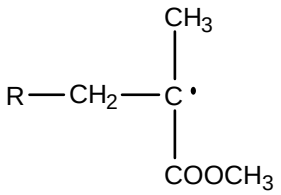

Fig. 1. Chemical structure of the polymerization radical in the PMMA bone cement formation.

applications in vivo: measurements of oxygen [10–12], pharmacokinetics of spin labels [13], detection of short-lived radicals by spin-trapping [14], formation of free radicals from drugs [15], formation of nitric oxide [16], monitoring of pH in tissues or in implants [17,18], detection of radicals in food [19] or in glass injuries [20]. The purpose of the present study was to demonstrate that it is feasible to noninvasively detect the free radicals generated during the bone cement formation using low frequency EPR. We used a commercial preparation of rapidly setting bone cement for bone surgery, compared the evolution in the number of radicals produced in vitro and in vivo after implantation in rats.

2. Materials and methods

2.1. Bone cements preparation

We used a commercial preparation of rapidly setting bone cement for orthopaedic surgery Palacos® (Schering-Plough). The methyl methacrylate was polymerized using a *N*,*N*-dimethyl-*p*-toluidine/benzoyl peroxide redox system. The commercial kit contains two parts: (1) powder containing methyl methacrylate/methyl acrylate copolymer (845 mg/g), benzoyl peroxide (5 mg/g), zirconium dioxide (150 mg/g), gentamycin sulfate (12.5 mg/g); (2) liquid containing methyl methacrylate (920 mg/ml, stabilized with hydroquinone), *N*,*N*-dimethyl-*p*-toluidine (20 mg/ml). A typical cement preparation consists in mixing 1 g powder and 0.5 ml liquid.

2.2. X-band EPR spectroscopy

EPR spectra were recorded at 9.3 GHz with a Bruker EMX EPR spectrometer equipped with a variable temperature controller BVT-3000. The parameters used were: power 0.8 mW, center field 3320 mT, sweep scan 10 mT, 2048 points, scan time 5 s, modulation amplitude 0.4 mT, 20 scans accumulation. Immediately after mixing, the sample was inserted in a quartz tube. The sample was maintained at 37°C. The spectra were recorded every 2 min during the first hour, every 20 min during the next 6 h and, then, once a day for the rest of the experiment.

2.3. L-band EPR spectroscopy

We used an EPR spectrometer (Magnettech, Berlin, Germany) operating with a low-frequency microwave bridge (1.2 GHz) and an extended loop resonator [20,21]. The parameters used were: power 25 mW, center field 46.93 mT, sweep scan 12 mT, scan time 1 min, modulation amplitude 0.46 mT.

2.4. In vitro measurements

A first experiment consisted in the polymerization of the bone cement alone. This sample was studied by X-Band (9.3 GHz) and L-Band EPR (1.2 GHz) spectroscopy. The intensity of the center-line was measured over time.

2.5. In vivo measurements

Rats were anesthetized with ketamine/xylazine ((8 mg/kg)/80 mg/kg, IP injection) and maintained with ketamine alone (injection 12 mg/kg each 10–15 min). In order, to implant the cement, the skin over the head was incised and the cement was placed on surface of the skull before suturing the wound. The head was placed under the extended loop resonator (1-cm depth sensitivity). 50 EPR spectra were accumulated (50 min acquisition). The first measurement was performed after 30 min, the second one after 5 h, and then, once a day. The intensity of the center-line was measured over time.

3. Results and discussion

A typical nine-line EPR signal, due to the interaction of the unpaired electron with neighboring methyl and methylene protons of the growing polymer chain was observed in the bone cements (Fig. 2). This was observed in vitro using X-Band (Fig. 2A) as previously described by other groups [5–9]. The amount of radicals produced in bone cements was sufficient to detect the EPR signal using lower frequency (L-Band) as shown in Fig. 2. This was observed in vitro (Fig. 2B), but also for the first time in vivo (Fig. 2C). As the kinetics of decrease of radicals takes several days, the acquisition time of 50 min is convenient to get in vivo an EPR spectrum with a good signal to noise ratio.

The generation and the decay of free radicals in the bone cements were studied by monitoring the variation of the EPR intensity signal over the time. The center-line was chosen because it possess the highest intensity. In Fig. 3, we present the evolution with the time of the EPR signal intensities recorded in bone cements in vitro and in vivo. In vitro, there is an initial short latent period (upper panel), followed by a very rapid radical growth associated with the polymerizing chain reactions, and a

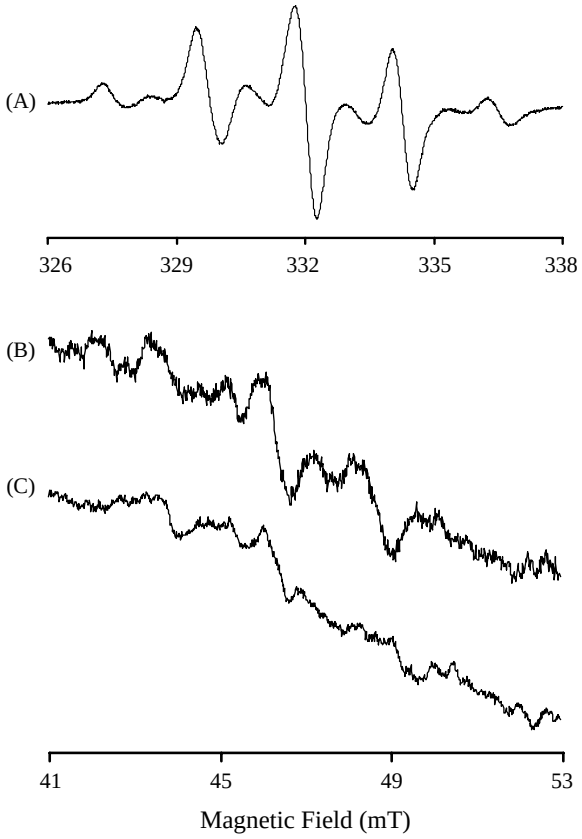

Fig. 2. EPR spectroscopy carried out on PMMA bone cements. (A) Typical EPR spectrum recorded in vitro using 9.3 GHz spectrometer. (B) Typical EPR spectrum recorded in vitro using an EPR spectrometer with a low frequency microwave bridge operating at 1.2 GHz and equipped with a surface coil resonator. (C): Typical EPR spectrum recorded in vivo in anesthetized rats using the same low-frequency spectrometer. Note the typical nine-line EPR signal due to the interaction of the unpaired electron with neighboring methyl and methylene growing polymer chain.

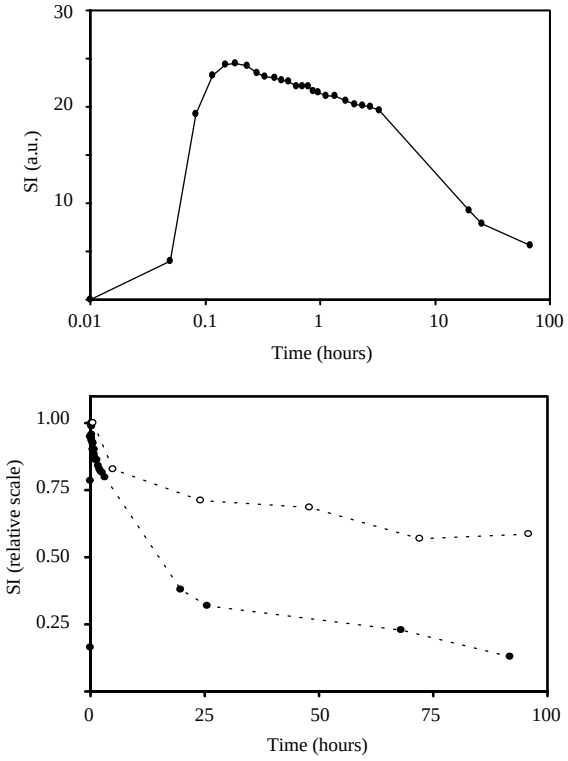

Fig. 3. Evolution with the time of the EPR signal intensities (SI) recorded in vitro and in vivo. Upper panel: Kinetics of generation and decay of polymerization radicals recorded in vitro. Note an initial short latent period, followed by a very rapid radical growth associated with the polymerizing chain reactions, and a long slow curing period during which the radicals disappear. Lower panel: Comparison of kinetics recorded in vitro and in vivo. Note the slower decay of radicals recorded in vivo.

Table 1
Polymerization radical decay rates in vitro and in vivo (mean ± standard deviation)

	Decay rate k_1 ($\times 10^5$) (s^{-1})	Decay rate k_2 ($\times 10^5$) (s^{-1})	n
In vitro	1.57 ± 0.46	0.25 ± 0.08	5
In vivo	0.51 ± 0.21	0.01 ± 0.007	4

long slow curing period during which the radicals disappear. These results are consistent with the ones previously obtained by Park [5]. In vivo, this lag time was not observed due to the timing needed for suturing the wound and placement of the animal in the magnet. However, Park previously demonstrated that this lag time is not significant for any practical applications [5]. It is more interesting to look at the decrease of the number of radicals as the amount of remaining free radicals is a marker of the amount of unpolymerized chains present in the polymer. Once the maximal amount of radical was reached, a bi-exponential decay of the number of radicals was observed, and decay rates (k_1 and k_2) of radicals were defined. The comparison of decay rates obtained in vitro and in vivo is shown in Table 1. Both decay rates k_1 and k_2 were significantly lower (t test, $p<0.01$) when the curing process occurred in vivo compared to the situation in vitro. These results are consistent with previous observations carried out ex-vivo on samples aged in animals [6]. As the kinetics are rather different in vitro and in vivo, this emphasizes the value of the method described here that permits the monitoring of the curing process directly in vivo.

In conclusion, direct observation of the free radicals produced during the PMMA bone cement formation was obtained for the first time in vivo using low frequency EPR spectrometers. Low frequency permits measurements in live animals due to the increased microwave penetration. The amount of polymerization radicals was carried out noninvasively over days on the same animals. This monitoring of the amount of unpolymerized chains present in the polymer allows to evaluate the state of the curing process directly in vivo. This method should be especially helpful in evaluating

the timing of polymerization in new types of PMMA bone cements. Thanks to the development of lower frequency spectrometers, typically in the MHz range with a higher wave penetration (several centimeters), these methods could be applied to larger samples or even to humans [22].

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