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PAPER

Radical-based grafting of GMA on sutures of different nature†

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Irradiation of a number of different sutures largely employed in the clinical practice with either high energy electrons or with γ-rays followed by quenching with glycidyl methacrylate (GMA) conveniently led to derivatization through a radical-based process. The radicals involved were detected by means of ESR spectroscopy and were characterized on the basis of their ESR spectral parameters which were also found to be consistent with the hfs constants predicted by DFT calculations. Evidence of the GMA derivatization of the sutures was obtained *via* ^{13}C CP-MAS NMR spectroscopy, while its extent was evaluated gravimetrically.

Introduction

Since the early '80s, the problem of suture-induced infection has attracted the interest of researchers,[1] and in the mid '90s the effects of different type of suture materials have been systematically studied.[2] Since 1992, the term surgical wound infections (SWIs) has been replaced with surgical site infections (SSIs), a replacement that underlines the relevance of the problem.[3] Presently, SSIs are defined as infections occurring up to 30 days after surgery (or up to one year after surgery in patients receiving implants) and affecting either the incision or deep tissue at the operation site. Very recently the technology and prevention of SSIs have been reviewed,[4] along with SSIs epidemiology, microbiology, and prevention.[5]

In the last ten years, the problem of limiting SSIs has also been tackled through the introduction of self-protecting sutures, that is by imbuing with appropriated drugs some of the commercial materials that are in direct contact of wounds. From a survey of the recent literature, it can be concluded that commercial materials impregnated with bioactive molecules are active in reducing the number of post-treatment infections.[6–8] Thus several such materials, after having been widely tested, are now marketed, *e.g.* sutures impregnated with the antibacterial Triclosan (Vicryl® Plus), gauze imbued with chlorhexidine (Bactigras®), and collagen fleece loaded with the antibiotic Gentamicin (GentaFleece®).

It should however be noted that impregnation processes may involve complex chemical and/or physical methodologies, with requirements that are also dependent on the nature of the material to be treated. One major point to be emphasized, is the way in which the drug is bound to the medical material. Indeed, binding a bioactive molecule to a substrate with a "permanent" chemical bond, changes it into a "new" drug that must therefore undergo all the necessary tests from the registering authorities. If, on the other hand, the drug and the substrate are held together by "weak" interactions, *e.g.* electrostatic and Van der Waals forces or hydrogen bonding, the complex will release the original drug and/or exert a topic effect in its free and already registered form.

In this light we have recently become interested in the glycidyl methacrylate (GMA) functionalization of different medical materials, in the optics that the bound GMA pendants might be further manipulated in order to act as links towards bioactive molecules. Thus we have shown that cellulosic materials (*e.g.* gauze) can be successfully GMA functionalized *via* a radical-based procedure.[9,10] We also proved that following hydrolysis of the glycidyl residues to glycerol, the derivatized material was able to bind reversibly through "weak interactions" bioactive molecules (*e.g.* antibiotics) to exert a topic effect and/or to be released once on site.[11]

We herein report on an extension of our studies to a similar functionalization of sutures of different nature, as a first step to obtaining new sutures effective in the prevention of SSIs.

Results and discussion

In the present study we have focused our attention on the GMA functionalization of three absorbable sutures, *i.e.* Vicryl®, Maxon® and Monocryl®, and two not absorbable sutures, *i.e.* polypropylene and Ethilon®, in terms of the results of the GMA binding and of the ESR characterization of the radicals involved in the functionalization processes.

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† This work is dedicated to the memory of Athel Beckwith, a teacher and scientist from whom we learned how to study chemistry by example. His pioneering advances in radical chemistry laid the foundation for much of the current radical clock methodology.

As we have already shown in previous studies on cellulosic materials,[10] GMA functionalization can be readily achieved through a process whereby radicals created in a substrate are quenched by a GMA solution. With cellulosic substrates the radical centres were generated by irradiation with either high energy electrons or γ-rays, but also by the action of hydroxyl radicals generated in a typical Fenton reaction.

Because of the absorbable nature of some of the investigated sutures the Fenton reaction could not be used in this study, and therefore ionizing radiation was used as the sole mean of substrate activation. Before the quenching in GMA, the irradiated sutures were investigated by means of ESR spectroscopy in order to substantiate the radical nature of the process and to characterize the different radical intermediates involved.

The efficiency of GMA functionalization was evaluated through quantitative product studies and by solid state CP-MAS NMR spectroscopy.

ESR Experiments

Irradiation of all sutures always resulted in the formation of free radicals, as indicated by the intense ESR spectra exhibited by the samples. In all cases the spectra of the irradiated sutures were only slightly anisotropic. They were reproduced using a standard program for the simulation of the powder spectrum of a single radical and then different simulations were combined together in different percentage to account for the presence of more than one radical species in variable amounts. Despite the good quality of the simulations so obtained, the presence of minor amounts of additional species other than those indicated below for each suture could not be excluded but was nevertheless disregarded.

Vicryl® sutures. Vicryl® sutures were irradiated in an oxygen-free atmosphere either with accelerated electrons from a 12 MeV linear accelerator (200 and 400 kGy) or with the γ-radiation from a ^{60}Co γ-cell (10.5 kGy).

When put inside the cavity of an ESR spectrometer, all the treated Vicryl® sutures gave rise to the same spectrum, independently of the received dose, with slight intensity variations. Typical irradiated Vicryl® experimental and computer simulated spectra are shown in Fig. 1, while the spectral parameters obtained from the simulation are reported in Table 1. The shown simulation implies the simultaneous presence of two carbon centred radicals in substantially different amount. The former and most abundant (~ 83%) of these species exhibits coupling of the unpaired electron with one single hydrogen atom, while in the second (~ 17%) the unpaired electron is coupled with a methyl group. As Vicryl® is obtained *via* the copolymerization of glycolic acid (90%) and lactic acid (10%), we envisage radicals **A** and **B** (Scheme 1) as the two carbon-centred radicals responsible for the observed ESR signals.

DFT calculations at the B3LYP/6-31G** level were carried out on model polymeric (trimers) radicals structurally related to **A** and **B** and the results are collected in Scheme 1. As it can be seen, in addition to the large single hydrogen and methyl splittings in **A** and **B**, calculations also predict smaller couplings for the hydrogens of the nearby but not adjacent methylene and methyl groups. While these smaller splittings are not experimentally observed due to the fairly large intrinsic line-width of the spectra, the very good agreement between the main experimental splittings and the calculated values strongly supports the radical identification.

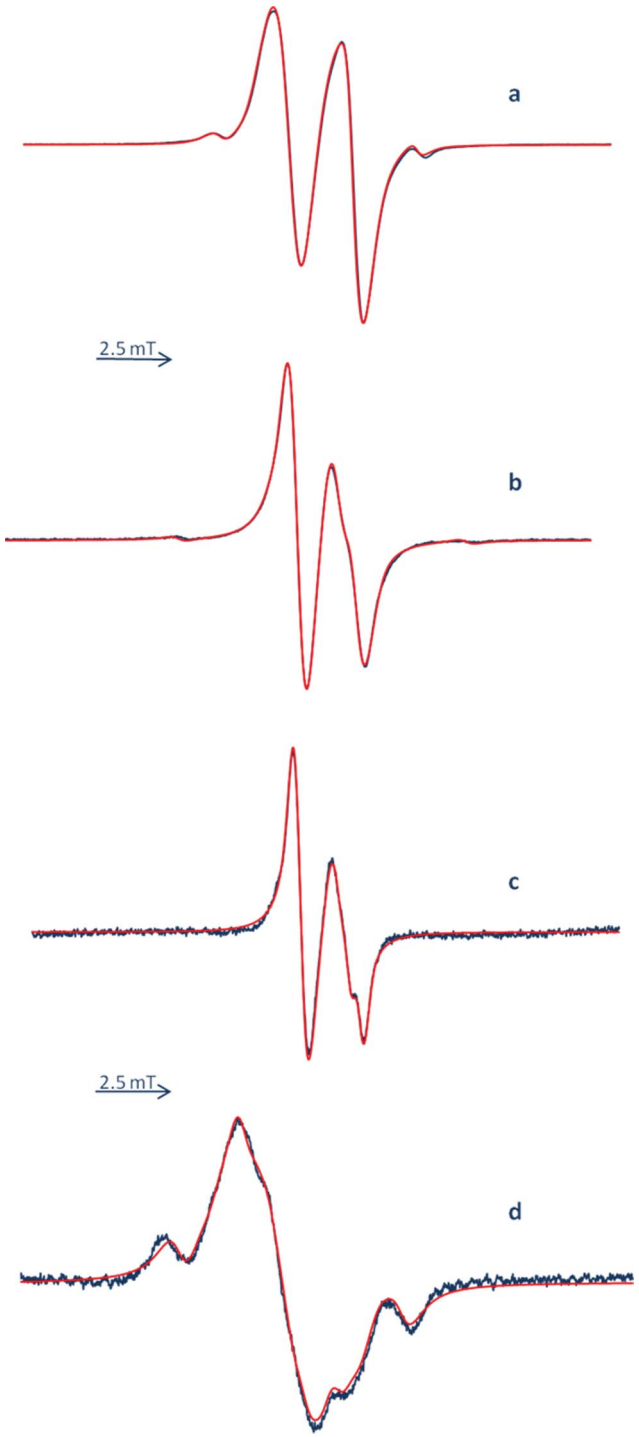


Fig. 1 Experimental (blue) and simulated (red) room temperature ESR spectra observed following high energy irradiation (200 kGy) of sutures: a) Vicryl®; b) Maxon®; c) Monocryl®; d) Ethilon®.

Finally, while the slightly larger percentage of radical **B** with respect to that of the corresponding monomer may be possibly attributed to a different stability of radicals **A** and **B**, the excess in percentage is so small that it can be disregarded.

Maxon® sutures. Maxon® sutures were administered doses of either 200 and 400 kGy (E.Beam) or of 10.5 kGy (γ-cell) in an oxygen-free atmosphere. In both cases irradiated samples

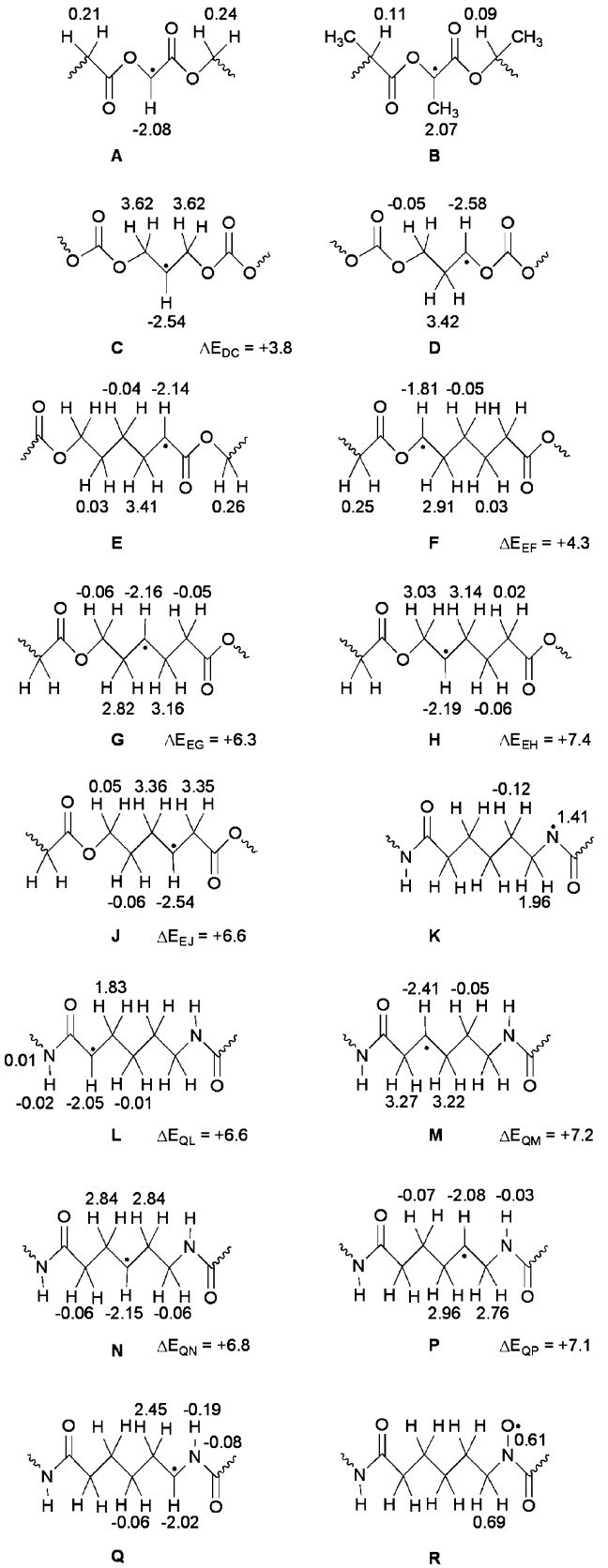
Table 1 ESR isotropic spectral parameters for the radicals detected following irradiation of different sutures

Radical	Hfs constants/mT	g
A	$a_{H\alpha} = 2.006$	2.0029_0
B	$a_{3H\beta} = 2.115$	2.0023_0
D	$a_{H\alpha} = 2.987$, $a_{2H\beta} = 2.955$	2.0036_0
L or **Q**	$a_{H\alpha} = 1.868$, $a_{2H\beta} = 2.500$	2.0027_7
K	$a_N = 1.490$, $a_{2H\beta} = 2.035$	2.0034_3
R	$a_N = 0.700$, $a_{2H\beta} = 0.844$	2.0058_0

led to the detection of identical ESR spectra, but for a different intensity of the signals. Typical irradiated Maxon® experimental and computer simulated spectra are shown in Fig. 1, while the spectral parameters obtained from the simulation are reported in Table 1. Also in this case the simulation was obtained assuming the presence of two different radical species in different amounts, one of which was the same already observed with Vicryl® and assigned to the radical with structure **A** (≥ 90%). Being Maxon® obtained through the copolymerization of glycolic acid (67.5%) and a trimethylene carbonate (32.5%), the two different structures **C** and **D** can be reasonably hypothesized for the second radical.

The spectral parameters of the second radical indicate coupling of the unpaired electron with a single α-hydrogen atom and two other equivalent β-hydrogen atoms, and apply well to a species structurally related to **D**. Indeed in a radical like **C** the unpaired electron should be coupled to four equivalent β hydrogen atoms, in addition to the one on the α-carbon. As a matter of fact, DFT calculations (see Scheme 1) predict for radical **C** a fairly large coupling with the α-hydrogen and a larger coupling with the four β-hydrogens of the two methylenic groups. On the other hand, the spectral parameters calculated for **D** indicate a large coupling with two β-hydrogens in addition to the doublet due to the α-hydrogen atom. The computed values fit reasonably with those derived from simulation thus lending support to our assignment. Calculations also predict small couplings with the hydrogen atoms of the methylenic groups across the carbonate moieties, but also in this case the large intrinsic spectral linewidth prevented their detection. It should also be stressed that calculations predict **D** to be more stable than **C** by 3.8 kcal mol^{-1}, which might explain why only this species was detected, out of the two radicals possibly originating from trimethylene carbonate. In this context, it should be further noted that the amount of radical **D**, as evaluated from the simulation, is small compared to the amount of the corresponding monomer in the original copolymerization mixture. We attribute this finding to a significantly greater stability of radical **A** with respect to radical **D**, possibly because the former species is a sort of 3-oxallylic radical where the unpaired electron can be delocalized onto the carbonyl oxygen.

Monocryl® sutures. Monocryl® sutures were administered doses of either 200 kGy (E.Beam) or of 10.5 kGy (γ-cell) in an oxygen-free atmosphere. Also in this case both kinds of irradiation led to the observation of very similar ESR spectra which, on the other hand differed from those of Vicryl and Maxon® sutures due to a somewhat more pronounced anisotropy (see Fig. 1) of the signals, possibly reflecting a greater crystallinity of this material, and to the presence of a single radical species that we identify again with **A**.



Scheme 1 DFT calculated ESR hfs constants (mT) for the radicals likely to be originated in the irradiation of Vicryl®, Maxon®, Monocryl®, and Ethilon® sutures. For isomeric radicals the relative energy differences are also reported.

Monocryl® is obtained from the copolymerization of glycolic acid (75%) and ε-caprolactone (25%). The detection of one (or more) among radicals **E–J**, the spectra of which should be characterized by rather large overall splittings due to coupling of the unpaired electron with at least one α- and two or four β-hydrogen atoms (see Scheme 1), was therefore expected. In this light the detection of the sole radical **A** is a puzzling finding that we find difficult to account for. Actually, on one side, it is not justified on the basis of the relative proportions of the two monomers. In stability terms, on he other hand, among the above mentioned species, the one predicted to be the more stable, namely **E**, should be resonance stabilized to an extent similar to **A**, unless the specific structure of **E** prevents this stabilization.

Polypropylene sutures. Polypropylene sutures have been irradiated in an oxygen-free environment, the administered doses being 10.5 kGy (γ-cell) and 200 kGy (E.Beam). Radicals resulting from high-energy irradiation of polypropylene have been long since widely investigated.[12–16] We therefore will not enter into details of our ESR results on irradiated polypropylene, but will just mention that they fit with the published data for the carbon-centred radicals that are formed in the absence of oxygen and for the polypropylenperoxy radicals, which the carbon radicals are immediately converted to in the presence of even a trace amount of oxygen.

Ethilon® sutures. Ethilon® sutures were administered doses of 200 kGy and 400 kGy (E.Beam) or of 10.5 kGy (γ-cell) in an oxygen-free atmosphere with similar results. These sutures are made of polyamide 6 and the primary radical species that may originate following its irradiation include an aminyl and 5 different carbon centred radicals (Scheme 1). To satisfactorily simulate the spectrum exhibited by irradiated Ethilon (also shown in Fig. 1) we assumed the overlapping of the signals from three different species which were identified as the aminyl **K**, the acyl-nitroxide **R** and a carbon centred radical. On the basis of the spectral parameters expected according to their structure, radicals **M**, **N** and **P** have been ruled out. In principle **L** seemed the obvious candidate due to the stabilization deriving from the possibility of delocalization of the unpaired electron onto the carbonyl group, nevertheless DFT calculations indicated radical **Q** as the more stable, although with a ΔE_{QL} of only 0.2 kcal mol^{-1}. This energy difference is too low to provide a safe ground for a choice, and we therefore prefer to leave open the two possible alternatives. Altogether, however, the spectral parameters collected in Table 1 are substantially consistent with the structures proposed for these species and with the values predicted by DFT calculations (see Scheme 1), even if the *g*-value of the nitroxide **R** seems to be slightly on the low side. Not surprisingly, upon contact with ambient air the spectrum of irradiated Ethilon® was drastically modified, only the signal from nitroxide **R** remaining visible.

Derivatization with GMA

GMA functionalization was carried out for all the irradiated sutures with the exception of Maxon®, the clinical use of which is presently being dropped. The derivatization process consisted of two steps, whereby the free radicals formed on the sutures following E.Beam or γ-irradiation are quenched by GMA. The goal of the irradiation procedure is to generate on the sutures, in an environment that will not affect them, radicals stable enough to survive the subsequent handling but at the same time enough reactive to be quenched by GMA. The results obtained with all sutures indicated that upon irradiation radicals are formed in an amount increasing with the administered dose (ESR experiments), and that these radicals react with the acrylate double bond when dipped in a GMA solution. GMA derivatization was proved gravimetrically, and was found to be dose-dependent. In addition, ^{13}C CP-MAS NMR spectrometry allowed the detection of typical signals of the glycidyl isobutyrate fragments in the spectral range 10 to 60 ppm and at *ca.* 178 ppm (see Fig. 2). Several quenching conditions were tested in order to maximize derivatization without on the other hand significantly modifying sutures' properties. The best results were obtained using a 1 : 1 (v:v) *i*-propanol/water GMA solution for the quenching that was carried out at 80 °C for *ca.* 20 mins under a nitrogen atmosphere. Lower temperature resulted in a dramatic when not total decrease of derivatization, higher temperatures degraded the sutures, and longer quenching time did not result in an increase of the derivatization yield while inducing a stiffening of the material.

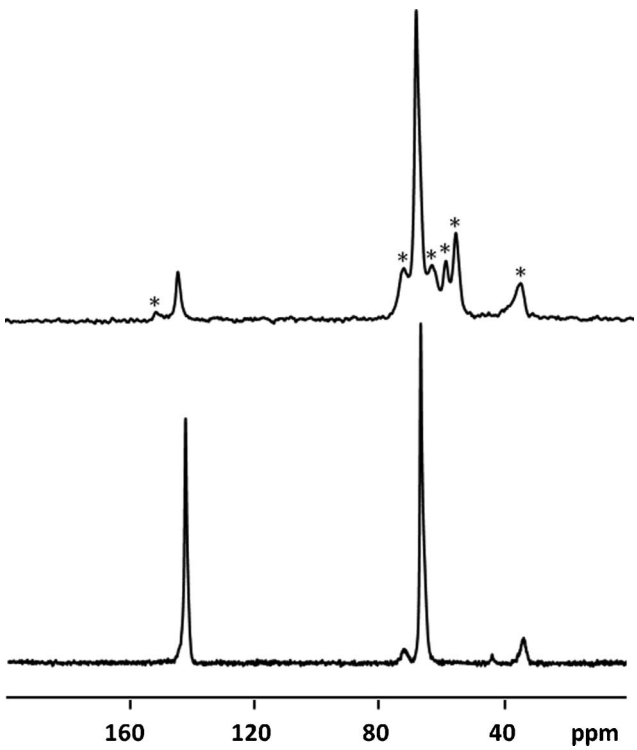


Fig. 2 Room temperature ^{13}C CP-MAS NMR spectra of Vicryl® as purchased (bottom) and after GMA derivatization (top). Asterisks mark the signals due to methylenic carbons of the glycidyl residues and of the homopolymer and of the *i*-butyrate methyl and carbonyl carbons.

From an examination of the data in Table 2, where the relevant results concerning the sutures' derivatization are collected, it appears that Vicryl® is by fair and large the more readily derivatizable substrate. Although the efficiency of the process was found to increase with the administered dose, the administration of 200 kGy increased the yield but was accompanied by an undesired stiffening of the suture. With Monocryl®, on the other hand, the

Table 2 GMA derivatization of different sutures

Suture	Irradiation type	Dose kGy	GMA [M]	T °C	Time min	Δweight (%)
Vicryl®	γ	10.5	2.45	60	60	—
Vicryl®	γ	10.5	2.45	70	40	0.67
Vicryl®	γ	10.5	2.45	80	25	114.46
Vicryl®	γ	10.5	2.45	80	40	117.52
Vicryl®	E.Beam	200	1.31	70	20	250.86
Monocryl®	γ	10.5	2.41	80	10	2.45
Monocryl®	γ	10.5	2.41	80	20	23.37
Monocryl®	γ	10.5	2.41	80	40	36.69
Monocryl®	E.Beam	200	1.27	70	20	14.20
Polyprop.	γ	10.5	2.45	80	30	32.89
Polyprop.	E.Beam	200	1.28	70	21	40.35
Polyprop.	E.Beam	400	1.28	70	21	27.05
Ethilon®	E.Beam	400	1.31	70	20	6.48

highest derivatization was obtained at low dose (γ-Cell), E.Beam irradiation resulting in a significant drop of the functionalization yield. Conversely, a 200 kGy dose proved to be the most effective in the treatment of polypropylene. The decrease of functionalization yield observed with Monocryl® and polypropylene at higher doses may reflect a degradation of the sutures, probably induced by the higher temperature. As for Ethilon®, derivatization was observed, although only to a fairly small extent, following administration of the highest dose. Overall it would appear that the low dose γ-cell treatment is sufficient for a satisfactory GMA derivatization only for the absorbable sutures, *i.e.* Vicryl® and Monocryl® whereas higher doses are preferable in the case of polypropylene and, even more so, of Ethilon®.

The very high sensitivity of polypropylene radicals to oxygen, as evidenced by the ESR detection of polypropyleneperoxy radicals also in some instances when oxygen "should" have been absent, let envisage the possibility that GMA moieties are grafted to the hydrocarbon backbone through an ethereal –C–O–C– bond rather than through a –C–C– carbon–carbon bond.[17] If this is the case, the monomer units of both polypropylene and polyGMA that are situated at the grafting site should have different signals in the CP-MAS spectra. These sites are however not statistically abundant, and the corresponding modified signals were not detected.[18] While we did not instead observe peroxy radicals with sutures other than polypropylene, whether the GMA moieties are grafted to these materials directly through a carbon-carbon or *via* an ethereal bond remains an open point.

Experimental

All sutures were commercially available, and were purchased from Ethicon (Johnson&Johnson). GMA and all solvents were of the purest commercial grade and were used as received.

Sutures irradiation

Samples of Vicryl®, Maxon®, Monocryl®, polypropylene and Ethilon® sutures were irradiated at ISOF Institute, Bologna, with γ rays from a ^{60}Co Nordion Gammacell 220 having a dose rate of about 7.5 Gy/min, and with electron beams from a 12 MeV Vickers Linac (most probable energy, 8.6 MeV; pulse length, 2 μs; pulse repetition rate, 50 Hz; dose per pulse, 6.2 Gy).

The samples of sutures were hermetically sealed under nitrogen atmosphere in small envelops made with a thin laminate plastic foil.

γ-Irradiations were performed by inserting the envelops containing the sutures inside a plastic phantom with wall thickness of 0.4 g cm^{-2}, suitable for establishing electron equilibrium. The dose rate of the Gammacell for the reference geometry was determined with the alanine reference transfer dosimeters from Risø High Dose Reference (HRD) Laboratory with an expanded uncertainty of 2.8% at $k = 2$.

Electron irradiations were performed with the envelops containing the sutures placed inside a plastic phantom (12 × 12 × 1.8) cm^3, at the same depth and position used for the dosimetry which was carried out using the super Fricke dosimeter solution. Traceability was established with the alanine reference transfer dosimeters from Risø High Dose Reference (HRD) Laboratory with an expanded uncertainty of 2.6% at $k = 2$. For each run, the phantom was placed in front of the accelerator output window in such a way that its central axis coincided with the beam axis. Good dose uniformity (≤ ± 3% over a 20 cm^2 area) was achieved at the location of the phantom with a 0.9 mm aluminium scatter plate.

All the absorbed doses referred in this paper (10.5 kGy for gamma irradiation and 200 and 400 kGy for electron irradiation) are absorbed dose to water.

After irradiation the bags were open inside a dry box filled with nitrogen. Small amounts of each suture were put in Suprasil® ESR tubes (i.d. 4 mm) that were sealed to avoid contact with air. The remaining part of each suture was weighted and then quenched with GMA.

ESR experiments

ESR spectra were recorded at room temperature by means of an upgraded Bruker ER200D/ESP300 spectrometer equipped with an NMR gaussmeter for the calibration of the magnetic field and a Systron-Donner frequency counter for the determination of *g*-factors that were corrected with respect to that of the radical cation of perylene in conc. sulfuric acid ($g = 2.00258$).[19] Typical spectrometer settings were: operating frequency, ~ 9.3 GHz (X band); scan width, 20 mT; scan time, 365 s; modulation amplitude, 0.1 mT; attenuation, 15 dB; receiver gain, 1×10^5 or as appropriate; number of averaged scans, 4 to 8. The spectra were simulated using a commercially available software or a software based on a Monte Carlo minimization procedure.[20]

DFT procedures

Unrestricted DFT calculations were carried out at the B3LYP/6-31G** level[21,22] to compute relative energies and hfs constants using the GAUSSIAN 03 system of programs[23] employing a valence double-ξ basis set supplemented with polarization functions (6-31G**).[24]

Quenching of irradiated sutures with GMA

A *i*-propanol-water solution (1 : 1 vol) of GMA was stirred for 30 mins at 80 °C under a nitrogen flux in a round-bottom flask. The irradiated material was then added to the solution and the reaction was kept thermostatted in a nitrogen atmosphere and

under stirring for about 1 h. The mixture was then cooled down, the solid material was recovered, exhaustively washed with water, *i*-propanol and acetone at room temperature and then kept in hot acetone for 1 h. The purified material was dried in air until a constant weight was reached.

Solid state ^{13}C CP-MAS NMR spectra

The ^{13}C cross polarization magic angle spinning (CP-MAS) spectra were recorded with a Bruker Avance 300WB spectrometer operating at 75.47 MHz. The following conditions were used: repetition time, 8 s; ^{1}H 90° pulse length 3.5 μs; contact time, 1.2 ms; number of scans, 2048 or 4096; acquisition time, 35 ms; spin rate, 8000 Hz. The materials were placed in a zirconium rotor, 4 mm in diameter and 21 mm high. The chemical shifts were measured relative to tetramethylsilane using benzene as secondary reference.

Conclusions

The present work highlights the possibility of functionalizing different sutures with GMA following irradiation with either γ-rays or energetic electrons. The ESR characterization of paramagnetic species after irradiation evidences the radical nature of the derivatization process. Because the investigated sutures appear to retain their physico-mechanical properties after functionalization, and because the glycidyl residue can be readily custom functionalized, the described procedure may open the way to new potentially useful materials.

Acknowledgements

This paper is dedicated to the memory of A.L.J. Beckwith, a friend and a great scientist. Without his unique contribution the present achievements of free radical chemistry would have hardly been possible.

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