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Multi-technique characterization of retrieved bone cement from revised total hip arthroplasties

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The purpose of this study was to assess the chemical composition, structure and degree of double bond conversion of retrieved bone cement from 29 total hip replacement revision arthroplasties, employing a multi-technique approach. Fourier transform infrared spectroscopy indicated that the retrieved bone cement samples were covered by a well-organized proteinaceous film rich in amides and alcohols, probably because of the adsorption of species from body tissues and fluids. X-ray fluorescence spectrometry showed the presence of potassium, sodium, calcium and phosphorus, implying the development of a mineralization process of the adsorbed biofilm. X-ray microtomography demonstrated a dense porous network in the bulk material comprised of macropores with a mean diameter > 1 mm. FTIR analysis of the degree of double bond conversion of retrieved samples was in the order of 70%, similar to that of samples prepared *in vitro* in air, but 30% lower relative to their counterparts mixed *in vitro* and set in water. Scanning electron microscopy revealed a porous cement surface, which replicated the characteristics of bone or femoral stem surface irregularities. The effect of the adsorption of species onto bone cement surface on the reactivity of the material with the surrounding tissues and materials, is currently unknown. The results of this investigation reveal that the *in vivo* aging pattern of bone cements may involve alterations, which cannot be simulated under current *in vitro* protocols, emphasizing the necessity for adopting *in vivo* approaches including retrieval studies in assessing bone cement properties.

1. Introduction

Bone cement is one of the most critical constituent arthroplasty components for the survival of total hip replacement because of its reactivity with the surrounding tissues and materials, as well as its involvement in two interfaces comprised of materials with dissimilar properties, that is metallic femoral stem and cortical femur [1–4].

A wide array of methods has been employed in the investigation of bone cement properties in various conditions [5–8]. The resultant multiplicity of the protocols employed for the investigation of nominally identical variables along with the absence of a reliable means to simulate the clinical conditions, have contributed to a dispute in the relevant literature concerning the clinical relevance of the results obtained from various methodological approaches [9]. Thus, various mixing regimes have been tested. These include (i) centrifuging at 500–4000 rpm, (ii) manual mixing with a spatula and variable frequency of strokes, or (iii) use of mechanical mixers with cyclic or eccentric movement. Different aging regimes including (i) air, (ii) water, (iii) blood serum or (iv) implanted subcutaneously in different animals, have been applied to simulate the clinical situation. The foregoing considerations may explain the wide range of values obtained. These may vary as much as 50% for nominally identical cement formulations, for the same tests among different investigators [9]. Studies now indicate that bone cement is exposed to stresses one or two orders of magnitude higher than was previously estimated because of the dynamic status associated with the range and intensity of movements [1]. These may also have a cumulative action [10], further contributing to the lack of conclusive evidence on this issue.

Aging of the material may take place at different stages of preparation and placement in the femoral cavity. Storing in light areas or at body temperature increases the brittleness compared to room temperature [11], whilst gama ray-sterilized bone cement samples have shown decreased fatigue strength relative to sterilization with ethylene oxide [12].

Immediately following placement, the presence of

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TABLE I Clinical information for the retrieved bone cement included in the study

Sex	Age	Weight (Kg)	Indication for primary surgery	Months in place	Indication for revision
F	60	78	RA	48	L
F	65	76	RA	98	L
M	67	89	F	46	AL
M	69	78	RA	48	I
M	69	65	RA	36	L
M	69	78	F	28	L
F	70	76	F	46	P
F	69	100	IN	72	AL
M	67	90	IN	36	P
F	68	95	RA	72	P
F	71	88	RA	24	L
F	68	84	IN	79	L
F	67	67	F	110	AL
M	74	75	F	101	P
F	72	86	F	47	P
M	69	87	F	118	L
M	67	88	F	120	I
F	66	90	F	40	L
F	69	64	IN	120	I
M	67	68	F	140	P
M	61	75	F	62	P
M	67	78	F	65	P
M	62	74	F	48	L
F	73	79	F	58	L
F	64	72	RA	112	L
F	67	80	RA	72	L
F	70	71	RA	67	L
A	78	67	RA	65	P
A	70	65	F	56	I

RA – rheumatoid arthritis, IN – indiopathic necrosis, F – fracture, P – pain, L – loosening, I – infection, AL – aseptic loosening.

traces of blood arising from the preparation of the cavity, induces a decrease in the shear strength of the cement–bone interface which may reach 50% [13]. It may be worth noting that the study cited above involved exposure of bone cement specimens to blood post-polymerization, and thus the effect of blood exposure during setting is unknown.

Finally, the effect of aging of bone cement during its clinical service in humans has not been fully investigated. In contrast, many studies have looked upon the mechanical properties of bone cement implanted in various animals. These show that the three-point bending strength of the cement decreased following 12–26 months of implantation in rabbits' dorsum [14], whilst others have found small differences for cement implanted in dogs for 8 weeks [15]. Also, tensile and compressive strengths were lower for bone cement implanted subcutaneously in animals relative to controls [16, 17]. However, the conditions to which the bone cement was exposed in these studies bear little if any similarity to the actual clinical situation since the bone cement was not subjected to loading upon placement and for the entire period of implantation, as occurs *in vivo*; it set outside the human body in contrast to clinical conditions where it was allowed to set partially inside the cavity. Also bone cement specimens of fixed dimensions and shape were utilized. Hence, no information is available on the structural alterations of bone cement induced *in vivo*.

The hypothesis tested in this study was that the aging of bone cements *in vivo* substantially alters the chemical composition and physical properties of the material. Therefore, the purpose of this investigation was to assess qualitatively and quantitatively the chemical composition and degree of double bond conversion of retrieved bone cement.

2. Materials and methods

The bone cement samples used in the study were collected during the revision surgery of 29 patients with total hip replacement at the Trauma Rehabilitation Hospital in Athens between 1997 and 1999. The bone cement preparation of the primary surgery included manual mixing using a spatula. Table I lists the population demographics as well as clinical information for this sample.

Explanted bone cement particles from each surgery were collected during the first 24 h following surgical retrieval. They were sterilized in ethylene oxide at 37 °C, number coded, and photographed [18]. Four bone cement samples facing the bone or the femoral stem with relative flat surfaces were selected from each retrieval case to facilitate increased resolution of the analytical methods used. These specimens were then subjected sequentially to multi-technique characterization within 48 h from retrieval.

2.1. Surface composition and characterization of integuments

The molecular composition of the integuments formed on retrieved bone cement surfaces was studied with micro-multiple internal reflectance Fourier transform infrared spectroscopy (micro-MIR FTIR). Spectra acquisition was performed on an FTIR spectrometer (PE 1760 X, Perkin-Elmer Corp., Norwalk, CT, USA) equipped with a micro-MIR attachment operating under the following conditions: 4000–400 cm^{-1} range, 4 cm^{-1} resolution, 50 scans co-addition, KRS-5 minicrystal of 45° edge and 14 internal reflections and 2.5 μm depth of analysis at 1000 cm^{-1}.

2.2. Elemental composition

X-ray fluorescence spectrometry (XRF) was used to identify the elemental composition of specimens using an energy dispersive XRF spectrometer (DX-95, EDAX International, Mahwah, NJ, USA) operating with a molybdenum Kα tube at 30 kV, 130 μA, low vacuum (–500 mm Hg), and a 10-mm collimated beam directed toward each specimen's surface at a 45° incident angle.

2.3. Bulk porosity and structure

High resolution X-ray microtomography (Skyscan 1072, Aartselaar, Belgium) was utilized to acquire images of the bulk bone cement morphology and microstructure under the following conditions: W source, 80 kV, 100 μA, 5.9 s exposure per section, 24 μm cross-section distance step cross-section pixel size 12 mm cross-sectional distant step, and section width of 1024 pixels.

2.4. Degree of cure

The degree of double bond conversion of the retrieved material was determined with the use of an FTIR spectrometer (PE 1760 X) on a percentage basis using the tangent baseline technique. Aliphatic carbon double bond (C=O) vibrations at the 1638 cm^{-1} area was chosen as an analytical frequency, whereas the ester bond (C=O) vibrations at the 1712 cm^{-1} area, which is not affected by the polymerization reaction, was utilized as a reference frequency. The% DC was estimated as:

$$\%DC = 100 \cdot [1 - (Ap_{C=C} \cdot Am_{C=C} / Am_{C=C} \cdot Ap_{C=O})]$$

where

$Ap_{C=C}$: the peak absorbance area of the set material at 1638 cm^{-1},

$Am_{C=O}$: the peak absorbance area of the unset material at 1712 cm^{-1},

$Am_{C=C}$: the peak absorbance area of the unset material at 1638 cm^{-1},

$Ap_{C=O}$: the peak absorbance area of the set material at 1712 cm^{-1}.

For the degree of conversion of bone cements set *in vitro*, ten rectangular specimens of 10 mm length, 4 mm width and 1 mm height were prepared from Palacos bone cement (Kulzer, Wehreim, Germany) according to manufacturer's instructions, which represented the vast majority of the retrieved bone cement samples, and allowed to set in air. Another set of ten identically prepared specimens were allowed to set with their top and bottom surfaces in contact with water to assess the effect of humidity on the polymerization efficiency of the material. The degree of cure of both groups was determined as previously. A fresh powder/liquid mix of the Palacos bond cement was used as an unset reference in all the conversion measurements.

The statistical analysis of the degree of cure results for the groups of bone cement set in air and water was performed by one-way ANOVA and the Tukey multiple comparisons test at $\alpha = 0.05$ level of significance.

2.5. Morphology and microstructure

Scanning electron microscopy was employed to assess the micro-morphological changes induced on retrieved cement surfaces facing the bone or the femoral stem. For this purpose, specimens were vacuum coated with a thin layer of conductive carbon and examined under an SEM at 15 kv accelerating voltage (JXA 733 Superprobe, JEOL Ltd., Tokyo, Japan).

3. Results

3.1. Surface composition and characterization of integuments

Fig. 1 depicts the FTIR spectra of a Palacos bone cement, which represented the vast majority of the retrieved samples, set in water and air. Fig. 2 illustrates representative FTIR spectra measured over 4000–450 cm^{-1} and shown expanded for (2000–450 cm^{-1}) of a retrieved bone cement specimen facing the stem. For the retrieved specimens, the following peaks are identified: –OH at 3300 and 1642 cm^{-1}, N–H at

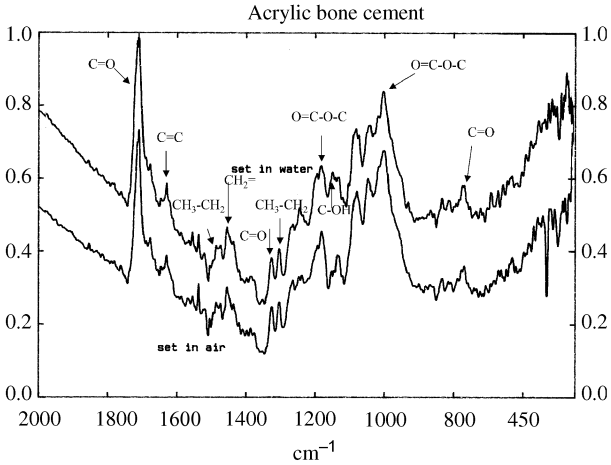

Figure 1 Micro-MIR FTIR spectra of non-implanted Palacos acrylic bone cement set in water (top) and air (bottom) (2000–450 cm^{-1} expanded region).

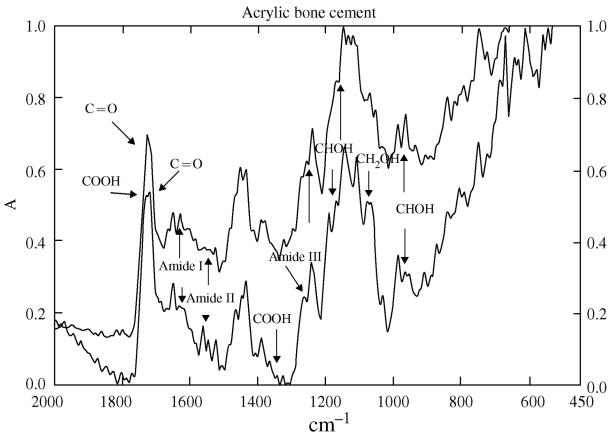

Figure 2 Micro-MIR spectra of retrieved bone cement specimens. Note the strong contribution of irreversibly adsorbed blood components assigned to amide I (1650 cm^{-1}), amide II (1540 cm^{-1}), amide III (1250 cm^{-1}), CH-b (1450 cm^{-1}), and C–OH (1100 cm^{-1}) groups. Minor peaks of COOH groups are detected as well.

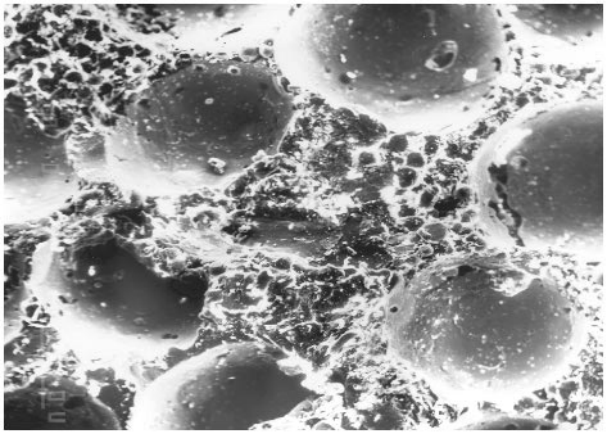

Figure 3 Secondary electron image (SEI) of a retrieved bone cement sample, which was in contact with the femoral stem, where the cement surface morphology replicates the porous coating micromorphology (craters) (160× magnification).

3200 cm^{-1}, $-CH_3$ at 2890 cm^{-1}, $-CH_2-$ at 2840 and 1380 cm^{-1}, atmospheric CO_2 at 2363 cm^{-1}, C=0 at 1712, 1320 and 820 cm^{-1}, amide I –CONH– at

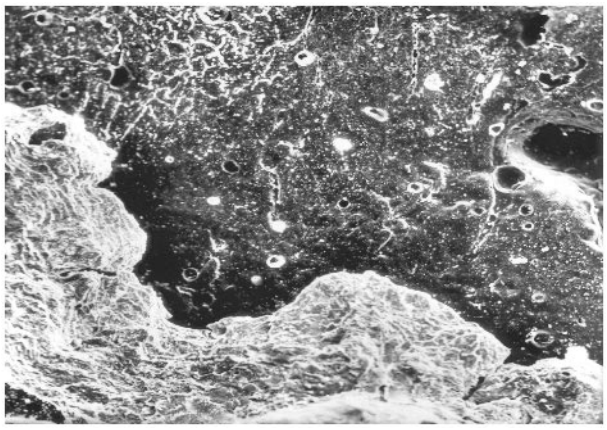

Figure 4 Secondary electron image (SEI) of a fractured part of a retrieved bone cement revealing bulk porosity (200 × magnification).

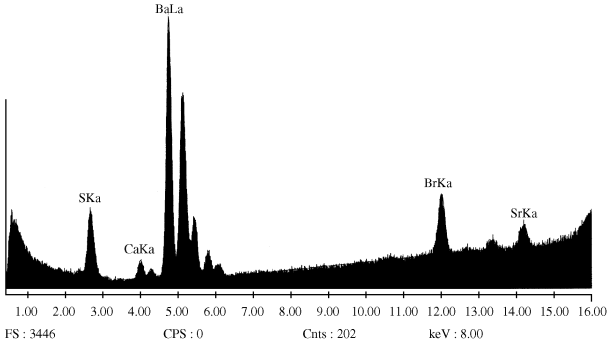

Figure 5 Representative EDXRF spectra of retrieved bone cement specimen.

1635 cm^{-1}, amide II at 1542 cm^{-1}, amide III at 1250 cm^{-1}, $–CO_3$ at 1450 and 870 cm^{-1}, –CH–OH at 1170 cm^{-1}, $–CH_2–OH$ at 1020 cm^{-1}, and $–PO_4$ at 960 cm^{-1}. The proteinaceous nature of integuments must be attributed to the adsorption of molecules from the surrounding tissues and fluids in the vicinity of the femoral prosthesis. The composition pattern shown was identical for all materials independently of retrievals.

3.2. Morphology and microstructure

In Fig. 3 the secondary electron image of a retrieved sample is depicted. The formation of crater-like depressions on the surface of the cement in contact with the femoral stem may be assigned to the replication of the porous stem surface. Fig. 4 illustrates the morphological condition of the retrieved bone cement demonstrating a porous surface with crack formation.

3.3. Elemental composition

Fig. 5 provides a representative XRF spectrum of a retrieved bone cement sample where the presence of peaks assigned to Zr, Sr and Ba are illustrated. Whilst these elements are constituent components of the cement, the presence of Ca should be assigned to adsorption and precipitations of calcium phosphate on the material. K, Na and Cl were additionally identified in other spectra of retrieved specimens.

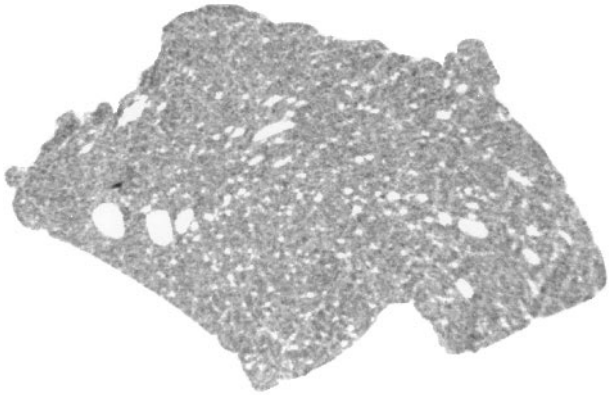

Figure 6 X-ray microtomography image of a retrieved bone cement sample illustrating substantial bulk porosity (white regions). Note the presence of three big pores at the left part of the image (26 × magnification).

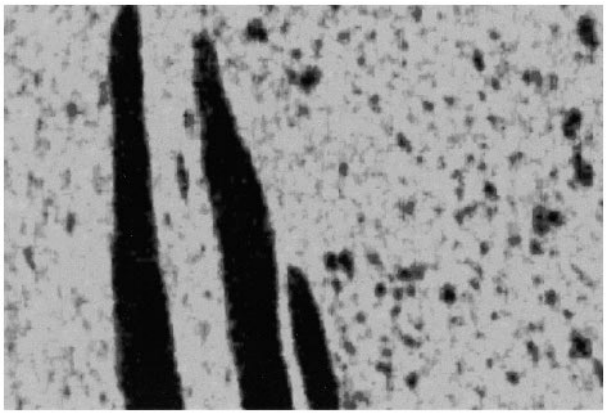

Figure 7 Two dimensional reconstructed X-ray microtomography image of the left region of Fig. 6 showing the extension of the pores through the entire specimen thickness (Inverse intensified image-pores are black, 60 × magnification).

3.4. Bulk porosity and structure

In Fig. 6 a representative high resolution X-ray microtomography image is shown. The increased porosity of the bulk material is apparent. It is interesting that the greatest pore sizes are located near the bone cement surface facing the femoral stem (left part of the image). Fig. 7 is the two dimensional reconstraction microtomography image through the entire sample at the regions corresponding to the big pores of Fig. 6. The image is in inverse grayscale to intensify the pore size by black color. These pores disrupt the integrity of the sample extending to the upper surface of the specimen. Macropores of size exceeding 1 mm and with a total volume in the range of 50% were observed for all retrieved samples. Table II provides the average pore size and percent in the bulk material of retrieved samples.

3.5. Degree of cure

The numerical data are provided in Table III where the retrieved specimens show a decrease in the order of approximately 30% relative to their counterparts set in water.

4. Discussion

Retrieval analyses have gained increasing interest in the broader implant biomaterials research since their intro-

TABLE II Pore size and characteristics of retrieved bone cement samples

Pore variable	Value (Mean)
Total volume	25.1 mm^3
Volume fraction	58.5%
Total surface	1250 mm^2
Surface-to-volume ratio	52.7 mm^{-1}

TABLE III Degree of cure (%) of bone cement set in air, set in water, and retrieved specimens

Group	Degree of cure Mean (SD)	Tukey grouping
Set in water	95.3 (4.2)	A
Set in air	69.6 (3.5)	B
Retrieved	69.8 (17.4)	B

duction by Hench [19], due to the critical information derived from investigating the performance of the material in the environment in which it was intended to function. The main factor, which distinguishes the *in vivo* environment from various storage media, is the presence of complex blood-derived substances along with local micro-environmental factors, which cannot be simulated under current *in vitro* research methodological approaches. Investigations of retrieved materials necessitate the use of a detailed clinical history along with information on the activity and life style of the patient.

In general, research in the field of bone cement has been structured around four basic axes:

1. *In vitro* study of mechanical properties employing a wire array of compositional and environmental factors [20–23]. Although this category represents the vast component of bone cement research efforts, it presents a striking clinical irrelevance due to the lack of the simulation of the clinical milieu in associated research protocols.
2. *Ex vivo* study of *in vivo*-aged samples, where polymerized specimens of bone cement are implanted subcutaneously or in the cavities of animals for a specific period [14–17, 24].
3. *In vivo* assessment of the prosthesis through imaging and X-ray techniques to provide information on the integrity of the interfaces formed, i.e. bone–cement and cement–stem [25, 26]. This method is most clinically relevant, nonetheless the means used do not provide any evidence on the mechanical or physical properties of the material. Further, the results obtained may be influenced by the limitations of the imaging or radiographic technique and the illustration of a three-dimensional object in two dimensions.
4. Retrieval studies, where the material is explanted during revision surgery or at autopsy [27]. This approach provides crucial information on the *in vivo* alterations of material, however most mechanical properties, which require specimens with fixed shape or dimensions, cannot be studied.

The organic functional constituents of the biofilm acquired on the bone cement examined in this study were mainly amide, alcohol, and carbonate whereas the predominating elemental species were Na, K, Cl, Ca, and P, and was identical for all samples. The elemental distribution of the biofilm is consistent with the formation of NaCl, KCl, and Ca-P crystalline precipitates [36].

Protein adsorption is initiated within a few milliseconds after contact with biological fluids [28]. The protein layers adsorbed are complex and may vary depending on the nature of the surface. In an aqueous environment, the interaction between hydrophilic surfaces with strongly attached water molecules and hydrated protein cores is weak, due to the development of hydrophilic repulsive forces [29]. Thus, on hydrated surfaces, hydrophobic and electrostatic interactions are expected to govern protein adsorption. Heavily charged macromolecules are probably the first species to be adsorbed, however, the first protein adsorbed does not determine the potential for attachment and the overall response at later stages. In most situations there is a competitive adsorption taking place with a rapidly decreasing protein affinity and increasing surface occupancy resulting in a sequential adsorption/desorption process of proteins, a phenomenon known as the Vroman effect [30, 31].

Adsorbed proteins may induce crystalline formation onto chemical groups, the stereochemical and surface charge of which allow for crystal nucleation. Extracellular non-collagenous proteins seem to be the principal components regulating crystal growth [31].

Protein-induced mineralization is a non-specific mechanism and proceeds by initial formation of KCl crystals, whereas calcium-phosphate precipitation may take place at later stages [32]. This mode of extracellular mineralization is involved in the formation of ectopic calcified deposits on tissues and materials including teeth [33], dental materials [34, 35], acetabular components of hip arthroplasty [36], and bioprosthetic valves [37], among others. Change in the surface adsorbed protein composition during the lifetime of a biomaterial's service may lead to utilization of different cell adhesion receptors, altering cell behavior. This is especially important for implantable devices, where an array of reactions related to tissue inflammatory response, activation of wound healing mechanisms and tissue remodeling takes place [38]. The presence of inflammatory cells at the interface initiates secondary reactions on biomaterial surface and the surrounding cell population by secretion of proteins, enzymes, oxidizing agents, and release of oxygen radicals [39]. These agents may change the biomaterial surface reactivity by either altering the types of proteins adsorbed onto or by affecting the structure of the original surface. The most common mechanisms of structural biomaterial modifications by biological fluids involve the near-surface regions and include absorption of biological species, desorption of ions, monomers, additives, impurities and release of breakdown products due to corrosion, wear or enzymatic attack [38].

The increased porosity of the retrieved samples may predispose to crack growth and propagation. Although some investigators have proposed that micropores with a nominal diameter of less than 1 μm, may act as crack inhibitors due to the consumption of the energy of the crack tip inside the pore [40], the pore size and distribution found for the specimens included in this

study, impose a high probability for catastrophic structural instability upon load application [41].

The degree of cure of the retrieved cement was in the order of 70%, not statistically significant different from setting in air. These values are significantly lower than the corresponding DC attained during setting in water. A possible explanation may pertain to the fastest diffusion of the residual monomer to the water than to the surrounding tissues at the vicinity of the implantation site after setting. This results in decreased availability of remaining monomer in the set material and thus in false increased curing efficiency. Considering the surface specificity of the micro-MIR technique used in the present study, the degree of conversion may not represent bulk cement conversion, but only conversion at the cement interfaces with air, water or tissues. Monomer leaching from poorly polymerized bone cement has been implicated in a wide array of biocompatibility problems through a direct and an indirect mechanism. First, methylmethacrylate may induce allergic reactions and triggering of histamine release mechanisms, which are associated with blood pressure fluctuations [42]. From a materials perspective, a less dense network as a result of the low degree of cure may lead to circulation of accelerators or polymerization inhibitors which possess a pronounced toxicity, which overcomes the toxicity of monomer itself [43]. Monomer monitoring during arthroplasty surgery has been found to reach levels as high as 7 μg/ml [44], and several precautions have been suggested by the FDA to protect the operating room team from exposure to this hazardous environment. Finally, a decreased degree of cure may adversely affect the performance of the material. A low degree of cure for a polymer is associated with compromised mechanical properties, physical instability and biocompatibility concerns.

Currently, there is a notable lack of information in the relevant literature, with only one study monitoring the degree of cure during surgery *in situ* [45]. However, no other study has investigated the aging process and the effect on degree of cure for periods as high as 120 months. Further research should address the alterations described in this study on the reactivity of the bone cement with its surrounding materials.

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