Assessing Environmental Effects on Organic Materials in Cultural Heritage: Chemical Deterioration of Artificially Aged Bone

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Keywords: environment, pollutants, cultural heritage, bone, ageing, analysis, chromatography, SEM, vibrational, spectroscopy, immunoassay.

Abstract

Under the auspices of INVENVORG (Thales Research Funding Program – NRSF), and within a holistic approach for assessing environmental effects on organic materials in cultural heritage (CH) artefacts, the effect of artificial ageing on elemental and molecular damage and their effects on the structural integrity of bone was investigated. Metapodial roe deer bone samples were artificially aged under humidity and atmospheres of sulfur and nitrogen oxides in room temperature. Elemental micro-analysis of bone material through SEM-EDX and molecular investigations through FTIR and Raman spectroscopy, high performance liquid chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELIZA) were realized. Results show damage within the inorganic and the organic matrix; incorporation of sulfur and nitrogen groups, minor reduction of specific aminoacids and changes in collagen integrity were evidenced. A synthesis of the various results is presented.

Introduction

Bone artefacts have enormous importance for our natural and cultural heritage, for understanding the past of life on earth and past human behavior. Bones also consist challenging objects for the conservator. The proper environment is essential for sustaining preservation of bone artefacts in museum exhibitions, archival repositories, etc. A wealth of information is available on the effect of burial conditions on bone artefacts (Child, 1995; Collins et al 1995; Stiner et al 1995; Smith et al 2007, Weiner 2010 and references therein). On the contrary, no comprehensive assessment of museum environmental conditions and their correlation to the various aspects of damage on bone has been undertaken.

An initiative focusing on a holistic approach for assessing environmental effects on organic material within cultural heritage (CH) artefacts has been undertaken in the form of INVENVORG (Investigation Of The Environmental Factors Effects On Organic Materials Constituting The Natural And Cultural Heritage) under the auspices of Thales Research Funding Program: Investing in knowledge society through the European Social Fund (Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework-NSRF). The results in this presentation are

part of this Programme. After a detailed experimental design (Dellaportas et al 2013), the congruous application of a wide range of techniques may offer valuable evidence as to the specific damaging level (mechanical, structural, molecular) through a systematic examination of factors like heat, humidity and gaseous pollutants (such as nitrogen and sulfur oxides) through artificial and natural ageing studies. Comprehensive damage assessment on other materials such as parchment has been previously reported [Della Gatta et al 2007, Odlyha et al 2007] and have been invaluable in providing information and guidelines for study methodologies, etc.

From the structural and molecular point of view, bone is composed of organic matrix (mainly collagen, with lower amounts of non-collagenous proteins, lipids and proteoglycans) and inorganic matrix i.e. hydroxyapatite and carbonates (Weiner 2010). Water is also essential (approximately 20%) part of bone, mainly as structural component (crystalline water), bound water, free water, and water (adsorbed in gaps of both inorganic and organic matrix elements (Timmins P. A. and Wall 1977, Gonzalez 2013).

Bone artefacts exposed to environmental conditions may suffer damages through exposure to light, moisture, heat, and atmospheric pollutants (such as sulfur and nitrogen oxides). The combined effect of these factors causes a complex deterioration pattern in the organic (mainly protein) and inorganic matrix. The chemical composition that reflects the molecular integrity of the complex bone matrix is affected. As a result of this, the color and surface morphology, and internal microstructure deteriorate. On the mechanical properties level, finally, collagen damage undermines the integrity of the material, making bone brittle leading to complete collapse. The state of preservation of the collagen fibers and that of carbonate hydroxyapatite are key targets for analysis at the molecular and elemental levels. This specific presentation focuses on artificially aged bone samples.

Results and Discussion

Bone samples and artificial ageing

Metapodial bone samples were taken from roe deer – *Capreolus* and were suitably processed to remove fat in room temperature without affecting the structure of the items. A universal laboratory (or artificial) ageing scheme has been designed, involving combination of factors like humidity (45 and 70 %RH) and atmospheres of certain concentrations of sulfur oxides (SOx, 100 and 300 ppm) and nitrogen oxides (NOx, 300 and 100 ppm) for 14 and 28 day periods. Temperature was kept at 25°C.

SEM-EDX analysis

In SEM-EDX analysis, randomly selected micro-areas on bone surface were examined and their elemental analysis was performed. Sulfur is clearly detected after exposure of the sample in SOx (with the presence of NOx) atmospheres. This demonstrates the effective incorporation of sulfur atoms inside the bone matrix in sulfur-polluted environments.

FTIR results

Previous investigations in collagenous materials, such as parchment aged in SOx and NOx atmospheres, have shown significant changes in FTIR spectra [Odlyha et al 2007, Della Gatta G., et al 2007). Similar observations were done in powder samples taken from the organic matrix of SOx and NOx-aged bones: moderate collagen removal (decrease in the ratio (A_{1668} / A_{1024}), suppression of amide II peak in relation to amide I, and newly-formed nitrate bands within the matrix were evident. Also, in the inorganic matrix, small decrease in carbonates type II (located at the trivalent PO_4^{3-} sites, detected as broad band at 1420 cm^{-1}) was detected. An extended array of spectra detected in artificially

aged samples in comparison with reference samples along with statistical evaluation of results are presented. Typical spectra are shown in Figure 1.

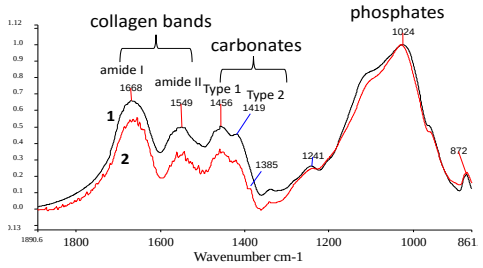

Figure 1: FTIR spectral region containing amide I, II, carbonates and phosphate groups of artificially aged bone sample.

Curve 1: Reference sample;
Curve 2: Aged sample.
Spectra in absorbance mode.

Raman Spectroscopy Results

FT-Raman spectra (1064 nm laserline of a Nd:YAG laser) showed peaks at ~1000 and 1045 cm^{-1}, indicative of the incorporation of sulfate and nitrate groups into the matrix, respectively. Changes to Carbonate/Phosphate, Phosphate/Amide I and Carbonate/Amide I ratios, which are the strongest predictors of mechanical properties of the samples, are moderate. A small reduction of the inorganic(BAP,960 cm^{-1})/organic(Collagen, 2940 cm^{-1}) ratio is indicative of reduced skeletal fragility and decrease of the corresponding Young modulus.

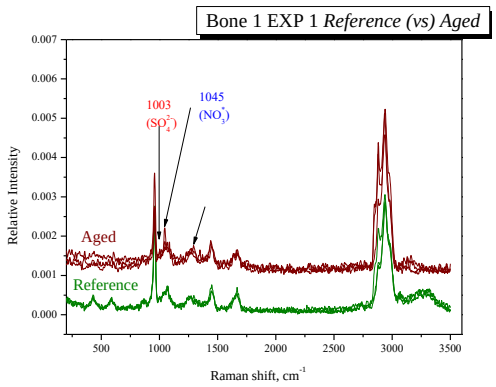

Figure 2: Representative right angle Stokes-side Raman spectra of reference and aged samples.

Green curve: Unaged (reference) sample; red curve: aged sample (Experiment 1 in Table I).

High Performance Liquid Chromatography in analysis of collagen and degradation products

Amino acid composition provides a 'fingerprint' of the molecular integrity of the polypeptide chains of collagen. Any changes in amino acid composition may reveal the presence of phenomena of oxidative or hydrolytic degradation of collagen in bones. Previous studies on parchment damage have showed that SOx and NOx gases interact with collagen molecules, which contained some amino acids particularly sensitive to the action of these pollutants (Della Gatta G., et al 2007). Certain aminoacids such as methionine, phenylalanine and tyrosine are known to react with these gases, but sulfur oxides are interfering suppressing the nitration of aromatic aminoacids.

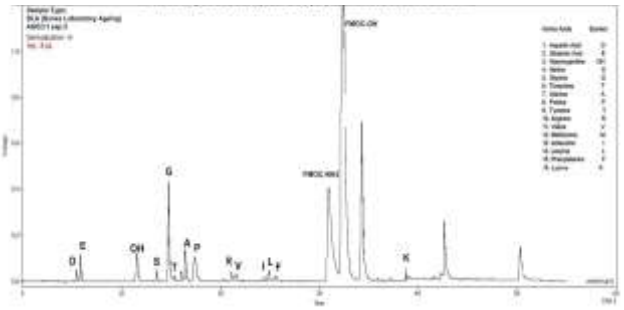

Figure 3: HPLC chromatogram of bone aged under a SOx (100ppm) and NOx (300ppm) atmosphere. Sample was HCl-hydrolysed and derivatized with FMOC (5mM).

The amino acid composition and content in bone collagen of artificially aged samples in SOx and NOx atmospheres was evaluated by calibrated amino acid analysis (typical chromatogram shown in Figure 3). No significant changes in amino acid composition of collagen were observed between the reference and aged bones, with the exception of small decrease of methionine, tyrosine and phenylalanine contents. Finally, free amino acids were detected in aged samples, mainly glycine, alanine, proline and hydroxyproline. Further investigations are under way.

ELISA assay

The Enzyme Linked Immunosorbent Assay (ELIZA) investigation is focused on the development of a reliable and reproducible immunoassay for the evaluation of collagen and keratin-based decay of organic materials in cultural heritage. For this purpose, polypeptide models of collagen, $[Pro\text{-}Ser(OBzl)\text{-}Gly]_n$ and keratin, $[Pro\text{-}Cys(Acm)\text{-}Gly]_n$, and Ac-YRSGGGFGYRSGGGFGYRS-βAla-NH_2, were synthesized and used in immunization experiments. The obtained antibodies were utilized in ELISA assays (absorption at 450nm) (Fotou et al 2012, Fotou et al 2014). According to our results, high recognition of the aged samples by the anti-polypeptide antibodies indicates significant deterioration, while high recognition of the anti-collagen and anti-keratin antibodies indicates lower deterioration.

Concluding Remarks

Artificially aged bone samples in atmospheric pollutants environment were analyzed with elemental and molecular analysis techniques. SEM-EDX microanalysis revealed sulfur incorporated in the case of aged samples. FTR analysis showed a small decrease in collagen content, incorporation of nitrates within the bone matrix, and no detectable crystallinity changes in the aged samples. The loss of molecular integrity was reflected through aminoacid analysis of the organic matrix with HPLC, which showed small decrease of certain aminoacids in the bone collagen, as well as through an ELIZA assay using anti-polypeptide antibodies.

Acknowledgements

The authors wish to thank Dr. L. Albu (ICCP – Romania) and Prof. D. Anglos and Dr. Z. Papliaka (IESL-FORTH) for providing access and assistance with their ATR-FTIR facilities.

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