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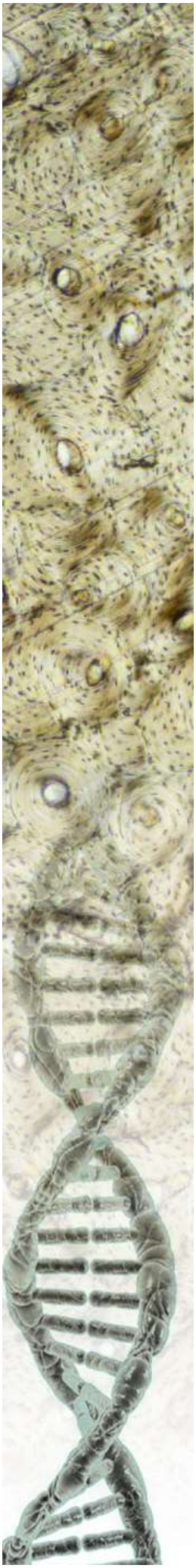


Ancient DNA Analysis from Epoxy Resin Biodur® Embedded Bones

Anna Lena Flux[1], Michael Schultz[2], and Susanne Hummel[1]

1. Introduction

For the microscopic investigations of the microstructure of archaeological bones, samples are commonly embedded in the epoxy resin Biodur® (cf. Hagens 1979) to determine the biological age at time of death of an individual or conduct histopathological investigations (e.g. Herrmann et al. 1990, Schultz 1988).

Although embedded samples are morphologically well preserved, the DNA extraction of this kind of sample material may be challenging because the embedding process may affect the DNA structure.

Hence, in the here presented study we tested whether Biodur® embedded bone samples may be a source of analyzable amounts of DNA.

2. Material & Sample Preparation

Material

six investigated subjects with two samples each:

a) bone samples of human femur diaphyses embedded in epoxy resin Biodur®

b) a sample of the respective native femur of each subject

Sample preparation

- the excess epoxy resin including the outer surfaces (contamination prevention) of the embedded bones were removed (cf. Figure 1, red dotted lines)
- respective native samples were taken from the femur diaphysis
- both sample types were incubated in 6% bleach, washed, and dried
- both sample types were milled to fine powder
- 250 mg powdered sample material – independent of whether it was pure bone material (native samples) or a mix of bone material and pulverized Biodur® that impregnated the bone during the embedding process – were subjected to the extraction

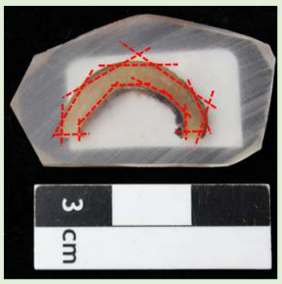
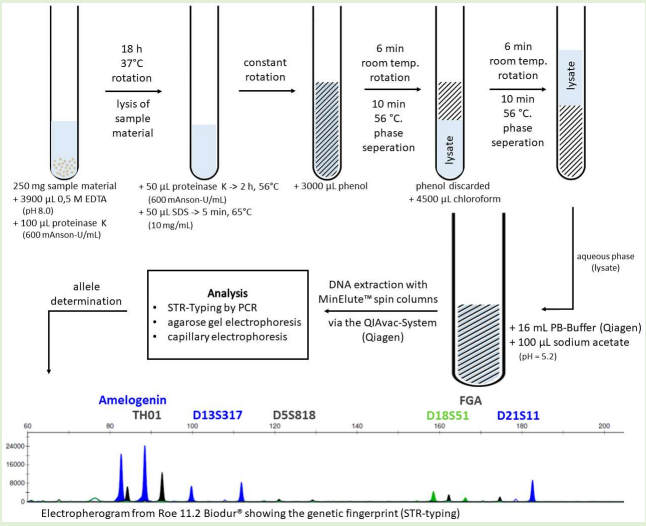


Figure 1: Sampling for embedded bone.

3. DNA Extraction & Analysis (e.g. Flux et al. 2017)



Electropherogram from Roe 11.2 Biodur® showing the genetic fingerprint (STR-typing)

4. Results

It was possible to extract and amplify analyzable amounts of DNA from both, the Biodur® embedded and the reference native samples (Flux, Schultz and Hummel in prep.).

- no major differences in DNA preservation between both sample types were visible with the exception of GS 53 (cf. Figure 2)
- differences in DNA preservation and amplification exist between the six investigated subjects, independent of whether the samples were embedded in Biodur® or native; only for Roe 12.2 the amplification failed almost completely for both sample types
- capillary electrophoresis and the STR allele determination resulted in full genetic fingerprints (cf. electropherogram in section 3) for all subjects with the exception of Roe 12.2

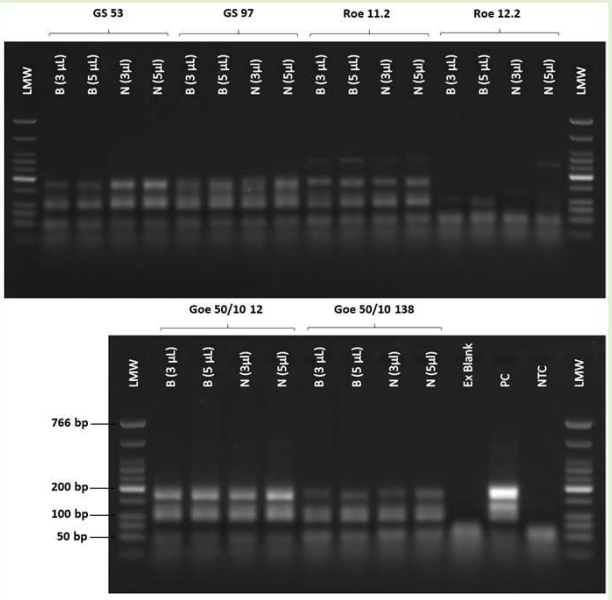


Figure 2: The results of the amplification of six autosomal STR markers and amelogenin for both samples, Biodur® embedded (B) and native (N) of the investigated subjects (DNA inset is bracketed). Gel bands are located between 75 and 200 bp of the DNA ladder. The extraction blank (Ex Blank) shows no bands. As a positive control (PC) contemporary DNA of the processor was used. For the negative control (NTC) water instead of DNA was used. Electrophoresis parameters: 8 µl PCR product with 2 µl Loading Dye, 3 µl size standard: Low Molecular Weight ladder (LMW, New England Biolabs® inc.), 2.5% gel, 110 V, exposure time: 0.4 seconds.

5. Discussion

Initially, we had expected that the Biodur® samples would reveal an inferior state of DNA preservation, since the chemicals of the embedding process or the Biodur® itself promoted DNA degradation. The results suggest that the Biodur® samples in relative terms even perform better than the native ones due to the fact that the 250 mg powdered sample material subjected to the extraction also contained Biodur® that impregnated the bone. The most plausible explanation for the outcome of the experiment is that powdered Biodur® is acting in a purifying way as, e.g., Chelex® 100 (Walsh, Metzger and Higuchi 1991) by binding proteins and humic acids, which would lead to more purified DNA extracts compared to the native samples. This would positively influence the efficiency of the PCR and in turn might compensate the lesser input of bone material to the DNA extraction.

Although the subjects Roe 11.2 and 12.2 are from the same burial site, the different amplification success is in full correspondence with the microscopic investigation of the respective thin sections from the bones (cf. Figure 3).

The authenticity of all results was proved by matching genetic fingerprints for both, the Biodur® embedded and respective native bone sample from one subject (e.g. Butler 2005).

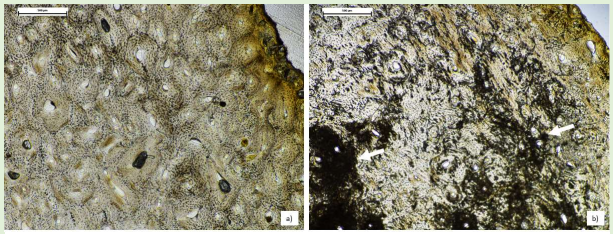


Figure 3: Microscopic pictures of thin sections (plain light, 120 µM) of the femurs' microstructures of the subjects Roe 11.2 (a) and Roe 12.2 (b). Different than in 11.2, the bone substance of subject 12.2 is severely damaged by microorganisms (arrows), which destroyed most of the organic compounds. Scale is 500 µm.

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