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Research Paper

Ethanol and formaldehyde fixation irreversibly alter bones' organic matrix

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ABSTRACT

Introduction: Biomechanical tests on bones are frequently accomplished in anatomically fixed tissues. The effects of ethanol or formaldehyde based fixation in bone material properties are subject to controversial discussions, regarding their appropriateness and usability to answer clinical questioning or biomechanical issues. We hypothesized that ethanol and formaldehyde irreversibly change bone material properties, and that this effect is mainly related to the bone's organic matrix.

Material and methods: Fixation related alterations in material properties were investigated in six fresh and two macerated human coxal bones by means of three-dimensional laser vibrometry based modal analysis. Ethanol or formaldehyde fixation were performed in one macerated and three unfixed specimens each. Changes in specimen weight and modal frequencies related to fixation, rinsing and drying were obtained. Modal assurance criterion (MAC) values were recorded to determine altered bone anisotropy.

Results: Due to fixation, modal frequencies were irreversibly altered in unfixed specimens, indicating weight loss in ethanol and structural changes in formaldehyde fixed specimens. In the macerated and inorganic controls, fixation related weight and modal frequency changes were reversible by rinsing. In the unfixed specimens, bone anisotropy was irreversibly altered by both modes of fixation, whereas the fixation related changes in bony anisotropy were reversible in the macerated controls after rinsing.

Discussion: Anatomical fixation that includes ethanol or formaldehyde irreversibly alters material properties of unfixed bones and impacts bone anisotropic properties, caused by changes in the organic matrix. In macerated bones that exclusively consisted of inorganic mineral salts, the observed effects on material properties and anisotropy were reversible. Conclusively, anatomical fixation on basis of ethanol or formaldehyde cannot be recommended, if material characteristics close to the vital state are of interest. Modal analysis

Abbreviations: MAC, Modal assurance criterion.

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is a potential method to gain insight into material properties, revealing the influence of the organic bone matrix on coxal bone elasticity.

1. Introduction

Material testing of biological tissues is of principle interest to deepen our understanding on human biomechanics in the healthy and in pathologically altered conditions. With increasing time it becomes increasingly difficult, if not impossible, to obtain reliable values from bones because of autolysis, bacterial contamination (Kääb et al., 1998), or by drying phenomena (Arnold et al., 1975).

To overcome this issue, e.g. when the specimen removal and testing take place at different places or extend over a longer period of time, freezing or anatomical fixation are performed (Topoliński et al., 2011). For anatomical fixation, ethanol and formaldehyde are commonly used (Blum, 1896; Romeis, 1989; Wilke et al., 1987) and also found in mixtures (Hammer et al., 2012; Messmer et al., 2010; Thiel, 1992). The effects of fixation on the tissues' biomechanics and their reversibility are still subject of controversial discussions, especially in bones: as a widely postulated statement, material testing of unfixed tissues is the gold standard. Some studies showed a clear impact of ethanol and formaldehyde fixation (Burkhart et al., 2010; Ohman et al., 2008; Wilke et al., 1987) on Young's modulus, the bending properties and the failure loads of bone. In contrast, other studies found negligible impact of anatomical fixation on the tissue characteristics even in the long-term use (Beaupied et al., 2006; Pöpperl et al., 1999; van Haaren et al., 2008). The influence of anatomical fixation on osseous region-dependent properties and anisotropy is still unknown to the authors' best knowledge. Also, it is unclear whether data from the unfixed state can be calculated from fixed specimens on the basis of conversion values. In order to give clear recommendations on whether anatomically fixed bones are appropriate for example to answer clinical questioning, it is essential to reliably determine the impact of anatomical fixation on human bones, especially with regards to their region- and direction-dependent properties (Topoliński et al., 2011). Furthermore, it is unclear if the organic matrix and the inorganic components are influenced differently by the fixatives.

Laser vibrometry based modal analysis has recently been introduced to obtain material properties of human bones (Bediz et al., 2010; Conza et al., 2007; Neugebauer et al., 2011; Skedros et al., 2006). This method allows gaining data on entire bones on basis of their mechanical and vibrational characteristics (Conza et al., 2007; Neugebauer et al., 2011). Additionally, data on region-dependent variations and anisotropy can be gained.

The aim of our study was to determine changes in the material characteristics of human bones related to anatomical fixation with ethanol or formaldehyde by means of modal analysis. The following issues were addressed: do ethanol or formaldehyde fixation irreversibly alter bone material properties and are these material properties related to the inorganic or to their organic matrix.

2. Material and methods

2.1. Human pelvis specimens

While alive, all donors gave their informed consent to the donation of their corpuses for research purposes. Eight human coxal bone specimens were obtained from body donors (mean age 75±8 years; four females and four males). The bones were completely removed from the right side of the pelvis and released from surrounding soft tissues including adjacent muscles, pelvic ligaments and the periosteum. For storage, the specimens were precooled at 3 °C before shock freezing them at −85 °C.

2.2. Maceration and anatomical fixation

Two specimens were macerated chemically to remove their organic components. Maceration was done by means of immersion for 24 h at 40 °C in a solution of a laundry detergent (Persil, Henkel AG, Düsseldorf, Germany; Elbroch, 2006). Rinsing of the specimens was performed with isotonic sodium chloride solution. Pure ethanol or 3 volume% phosphate buffered formaldehyde (Romeis, 1989) solutions were used for anatomical fixation. Three fresh and one macerated bone specimen each were fixed with ethanol or with formaldehyde by means of immersion. Modal values and specimen weights were obtained at a temperature of 23 °C and at an air humidity of 25%. The fresh specimens were investigated in the following order: the unfixed, followed by the ethanol fixed or the formaldehyde fixed and finally by the rinsed state. The two macerated specimens were investigated as follows: the initial dry state, followed by the initial rinsed, then the ethanol fixed or the formaldehyde fixed, the rinsed and finally the dried state. Each step of fixation and rinsing was performed for 24 h in darkroom environment to minimize potential effects of formaldehyde related decalcification due to light exposure. Drying was performed for twelve weeks.

2.3. Specimen weighing

Each of the specimens was drained for 15 min before the measurements. Then, their masses were recorded immediately before and after performing the modal analyses in each step. Both values were then averaged to minimize the error introduced by liquid loss during the measurement.

2.4. Modal analysis

Three-dimensional laser vibrometry was utilized to record the frequencies in response to mechanical excitation by means of an electro-dynamic shaker (Mini-shaker Type 4810, Brüel & Kjær, Nærum, Denmark) according to Neugebauer et al. (2011). A PSV-400 scanning vibrometer, combined