THE EFFECTS OF VARIED CONCENTRATIONS OF FORMALIN ON THE TENSILE STRENGTH OF CORTICAL BONE: SHOULD EMBALMED BONE EVER BE USED FOR BIOMECHANICAL TESTING

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**Purpose:** Despite the known effects of formalin fixation on the mechanical properties of bone, embalmed cadaveric specimens continue to be used in vitro biomechanical experimentation. The embalming effect has been presumed to be uniform, acting as its own control, enabling comparisons of relative strengths of constructs and implants. As local concentrations of embalming solution are dependent on vascularity and pooling effects that may differ between or within specimens, these biomechanical effects may not be as uniform as previously assumed. To study these effects, the authors compared the effects of various dilutions of formalin embalming solution on the tensile properties of bovine femoral specimens. The authors hypothesized that there is a biomechanical difference between bones preserved in full strength and dilutions of embalmed solution. This difference may detract from the validity of many biomechanical investigations using formalin-fixed bones.

**Methods:** Specimen preparation—Eight fresh bovine femoral shafts were osteotomized at their distal and proximal ends, leaving 8-cm sections of intact cortical specimen. Sections were then transected into anterior, medial, lateral, and posterior segments, and milled to a uniform 2.00-mm thickness. Using a custom hour-glass (ASTM-tensile standard) shaped router template, 32 bovine cortical specimens with identical dimensions were fabricated according to previous authors’ specifications. Bone hydration was maintained with calcium-buffered saline during the entire process. A standard formula embalming solution was prepared from equal parts of 10% formalin, buffered-saline, and 95% glycerine. Portions of this solution were then diluted with buffered-saline to form ½ strength and ¼ strength solutions. Prepared specimens were assigned to one of four groups, full strength, ½ strength, ¼ strength or unembalmed (fresh frozen), in a balanced complete block arrangement and kept frozen or immersed in their respective solutions for 120 hours at room temperature before processing with the chemicals. Fresh-frozen specimens were thawed in calcium-buffered saline six hours prior to testing.

Specimen testing: All specimens were tested to tensile failure using an Instron model 1321 servo-hydraulic materials testing apparatus at a strain rate of 2.65x10⁻⁷ %/sec. Force and displacements were recorded using the systems software and transferred to Microsoft Excel® for data analysis. The ultimate tensile strength and modulus of elasticity were calculated for each specimen and statistically analyzed for significant difference using a paired Student T-tests and a repeated measures analysis of variance between the four groups.

**Results** The average ultimate tensile strength for the unembalmed specimens was 271 MegaPascals. Specimens in the full strength solution averaged 324 MegaPascals, while specimens in the half-strength and quarter-strength solution averaged 332 MegaPascals and 290 MegaPascals, respectively. Statistical analysis revealed a significantly higher value of ultimate tensile strength and quarter-strength specimens (p<0.1). In addition, a significant difference existed between unembalmed versus half-strength specimens, as well as between unembalmed and full-strength groups (p<0.05). With the numbers available, there was no significant difference detected between full strength and half-strength or quarter-strength.

The elastic moduli were averaged and statistically compared. Unembalmed specimens averaged 24.3 GigaPascals, while full strength, half-strength, and quarter-strength averaged 26.8 GPa, 26.7 GigaPascals, and 24.2 GigaPascals, respectively. A paired T-Test comparing half-strength and quarter-strength demonstrated a statistically significant difference (p<0.1). Full strength and quarter-strength differed significantly, as did full strength and unembalmed bones (p<0.05). With the available numbers, there was no significant difference between full strength and half-strength groups. A repeated measures analysis of variance revealed no detectable differences between the elastic moduli of any of the groups with the available numbers.

**Discussion:** In the authors’ review of the literature, no previous investigation of the effects of differential concentrations of embalming fluid on cortical long bone specimens was found. The mechanical effects of full strength formalin-embalming solution on cortical bone are well-documented. Despite these findings, investigators have justified the use of embalmed bones for biomechanical study by assuming uniform biomechanical effects throughout the specimens. Conclusions from such studies are limited to relative differences between testing groups and rely on the assumption that formalin penetration is uniform in all specimens.

This assumption may not be valid. Embalming fluid is delivered to exsanguinated tissues through the vasculature, most commonly via the internal jugular vein, to mix with residual body fluids. More vascularized tissues have greater infiltration of embalming fluid. In addition, structures closer to the introduction site are subject to higher pressures of infiltration. With these factors, it is illogical to think that local formalin concentration would be consistent throughout a long bone, between contralateral long bones, or between long bones from different cadavers. The investigators chose a commonly used formula as the full strength concentration to represent zones of optimal embalming penetration, while dilutions of this solution were used to represent areas of decreased infiltration. The authors’ hypothesized that there is a detectable difference in the biomechanical properties of bones preserved in different formalin solution concentrations.

Mechanical testing of cortical long bone specimens is influenced by many factors, including both structural and material properties. The current study demonstrates statistically significant different material properties between cortical bone specimens preserved in varying dilutions of formalin embalming fluid in this standardized in vitro biomechanical model. In addition to inter- and intra-specimen variation of size, shape and density, this phenomenon may account for some of the variability observed in orthopaedic biomechanical studies performed using embalmed cortical long bones. Use of fresh frozen unembalmed bones is preferable and remains the gold standard for in vitro orthopaedic investigation. Although formalin-fixed specimens are less than optimal, the authors suggest immersion of specimens in a standardized formalin solution may minimize the non-uniformity of the embalming process yielding a more consistent biomechanical medium. Without this technique, embalmed cadaveric bones should not be used for biomechanical investigation.

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