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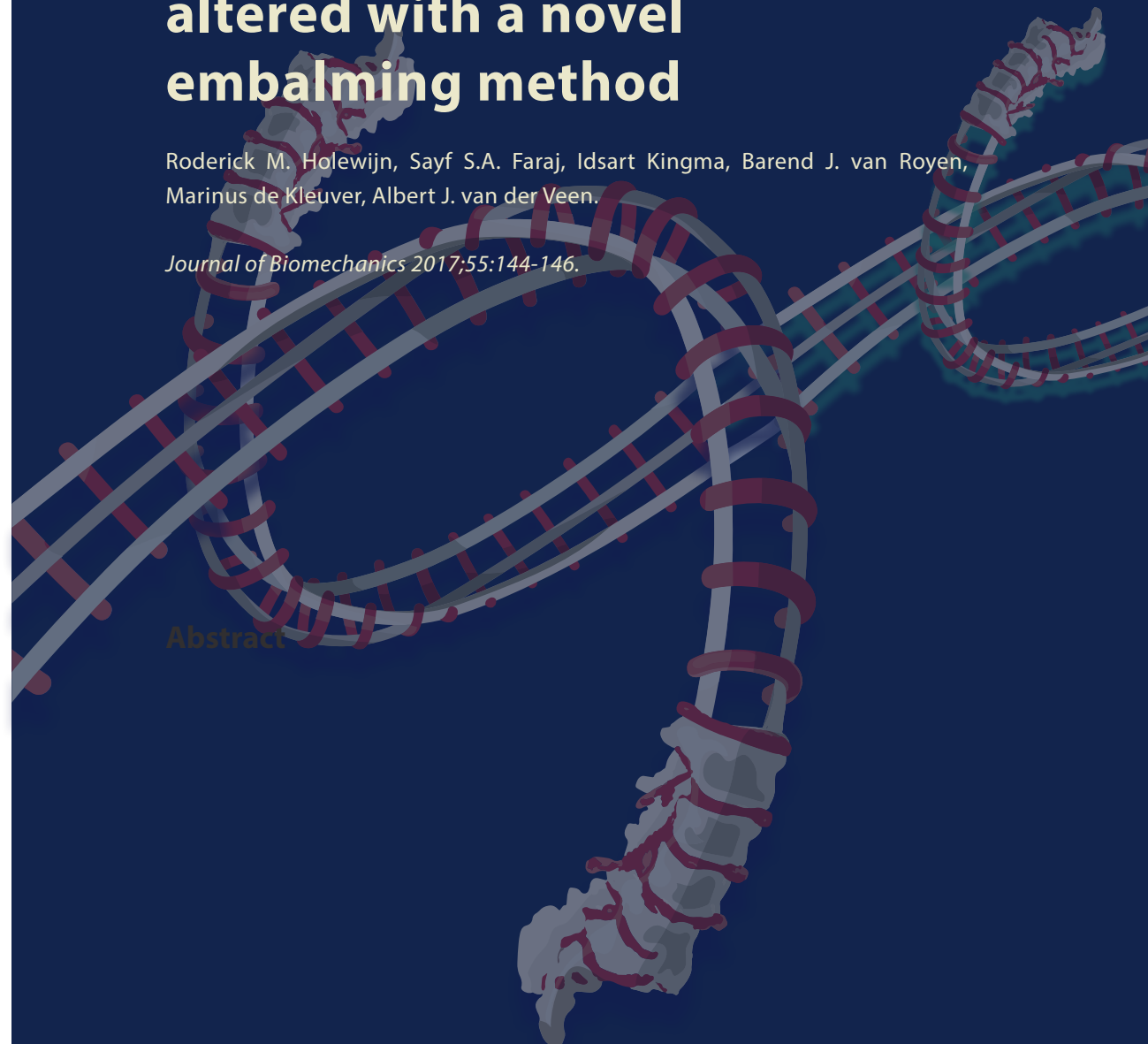
Chapter 3

Spinal biomechanical properties are significantly altered with a novel embalming method

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Abstract



Abstract

In vitro tests on the biomechanical properties of human spines are often performed using fresh frozen specimens. However, this carries the risk of pathogen transfer from specimen to the worker and the specimens can only be used for a limited amount of time. Human spinal specimens embalmed with formaldehyde carry an almost absent risk of transfer of pathogens and can be stored and used for a long time, but the tissue properties are strongly affected making this method inapplicable for biomechanical testing. In this study, a new embalming technique called Fix for Life (F4L), which claims to preserve the tissue properties, was tested. The range of motion (ROM) and stiffness of six fresh human spinal specimens was measured using a spinal motion simulator before and after F4L embalming. After F4L embalming, spinal stiffness increased in flexion-extension by 230%, in lateral bending by 284% and in axial rotation by 271%. ROM decreased by 46% in flexion-extension, 56% in lateral bending and 54% in axial rotation. In conclusion, based on this study, F4L does not maintain physiological spinal biomechanical properties, and we propose that this method should not be used for biomechanical studies. Nevertheless, the method may be an alternative to formaldehyde fixation in situations such as training and education because the effect on spinal biomechanics is less detrimental than formaldehyde and tissue color is maintained.

Introduction

In vitro cadaver tests using fresh frozen human cadaveric spinal specimens are performed to test surgical techniques or new spinal implants [1]. However, the accompanied risk transfer of pathogens is a significant downside of this method and fresh frozen tissue can only be used for a limited amount of time before autolysis sets in.

Embalmed human spinal specimens carry an almost absent risk of pathogen transfer and can be stored and used for a long time. However, to date, embalming is mostly performed using formaldehyde. This embalming method drastically decreases the spinal range of motion (ROM) by 80% in all loading directions, and the neutral zone by up to 96% [2]. An ideal embalming method should combine disinfection and fixation to prevent autolysis without affecting the biomechanical properties of the tissue.

This would greatly improve the possibilities for biomechanical experiments, which are of paramount importance in the preclinical evaluation of new spinal implants.

Recently, the novel embalming method Fix for Life (F4L) was presented [3]. This new embalming method uses a lower formaldehyde concentration. It is claimed that this new method preserves the consistency and flexibility of the tissue and is applicable for biomechanical testing [4]. To date, no biomechanical analysis of human cadaveric specimens embalmed with this new method has been performed. This study aims to analyze the effect of F4L embalming on the biomechanical properties of human cadaveric spines.

Methods

Six human thoracic spinal segments (T6-T11) were harvested from fresh frozen human cadavers. Excessive muscle tissue was removed and all ligaments and bony structures were left intact. The proximal and distal vertebral bodies were embedded in metal pots using a low-melting-point bismuth alloy (Cerrolow-147; 48.0% bismuth, 25.6% lead, 12.0% tin, 9.6% cadmium, and 4.0% indium)

The testing setup (Fig. 1) was described and validated previously [5]. In short, the pots were placed in a custom build spinal motion simulator and pure moments of 4 N m were applied using a hydraulic materials testing machine (Instron, model 8872; Instron and IST, Norwood, Canada). Before testing, a compressive axial preload of 250 N was applied for 1 h to obtain physiological conditions in the intervertebral disc. Biomechanical testing was performed inflexion-extension, lateral bending and axial rotation. During the experiments the room temperature was kept constant at 20° Celsius. Specific care was taken to keep the spinal specimens moist. Therefore, the fresh spinal specimens were regularly sprayed with a saline solution and the embalmed specimens with F4L preservation fluid.

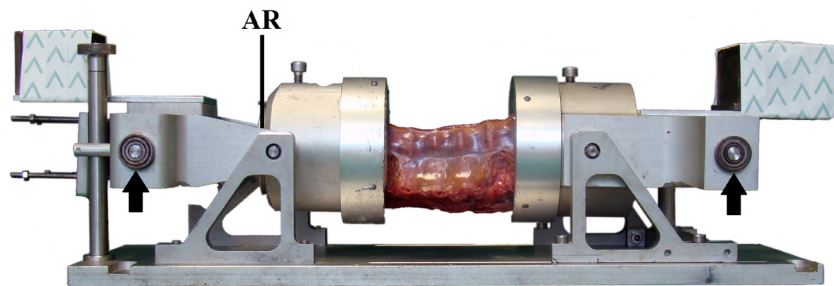


Fig. 1. The testing setup with the human thoracic spinal specimen (T6-T11) positioned in the spinal motion simulator. A materials testing machine applied loads to the two points designated by the arrows. To test lateral bending, the specimen was rotated 90°. For axial rotation, the left cup was rotated using a steel cable powered by the materials testing machine. Reprinted with permission from Elsevier [13]

The fresh specimens were tested first. Next, according to the manufacturer's recommendations, the specimens were immersed in a solution of 4 l of F4L embalming fluid, 18 l of water and 0.3 l of 37% formaldehyde for seven days. The specimens were then rinsed with demi water and immersed in F4L preservation fluid for four weeks.

Each loading direction was tested for three cycles and data of the third cycle was used for analysis. The range of motion (ROM) and the stiffness of the complete T6-T11 spine section was calculated from the load-displacement data of the testing machine. The ROM was calculated between -4 N m and +4 N m. The stiffness was calculated between -1 N m and +1 N m, in accordance with earlier studies [6]. The stiffness was measured using a least squares fit of a straight line through a fitted curve of the load-displacement data. The slope of this line represented the stiffness.

The effect of F4L embalming and subsequent preservation in F4L solution on the biomechanical parameters was tested using a two-side paired *t*-test. *P*-values below 0.05 were considered statistically significant.

Results

The effect of the F4L embalming technique on macroscopic spinal tissue morphology is demonstrated in Fig. 2. It can be observed that the color remained fairly identical after embalming. Fig. 3 shows that the load deformation curves demonstrate no hysteresis after embalming. The F4L embalming method had a large and statistically significant effect on the ROM and stiffness in all loading directions (Table 1). Stiffness increased in flexion-extension ($230\% \pm 133\%$) (mean \pm SD), lateral bending ($284\% \pm 65\%$) and axial rotation ($271\% \pm 73\%$). This was accompanied by a decrease in ROM in flexion-extension ($46\% \pm 6\%$), lateral bending ($56\% \pm 4\%$) and axial rotation ($54\% \pm 5\%$).

Table 1. ROM and stiffness (mean \pm SD) for fresh and subsequently Fix for Life embalmed thoracic spinal specimens.

	ROM (degrees)	Stiffness (degrees/Nm)
Flexion-extension		
Fresh	8.3 ± 3.5	0.8 ± 0.5
Fix for Life	3.8 ± 1.2	1.9 ± 0.8
P-value	0.012	0.004
Lateral bending		
Fresh	10.5 ± 2.9	0.4 ± 0.2
Fix for Life	5.9 ± 1.7	1.2 ± 0.4
P-value	0.001	0.004
Axial rotation		
Fresh	20.8 ± 6.6	0.3 ± 0.1
Fix for Life	11.2 ± 3.0	0.7 ± 0.2
P-value	0.005	0.001

Results presented as mean \pm SD for fresh and subsequently Fix for Life embalmed thoracic spinal specimens.

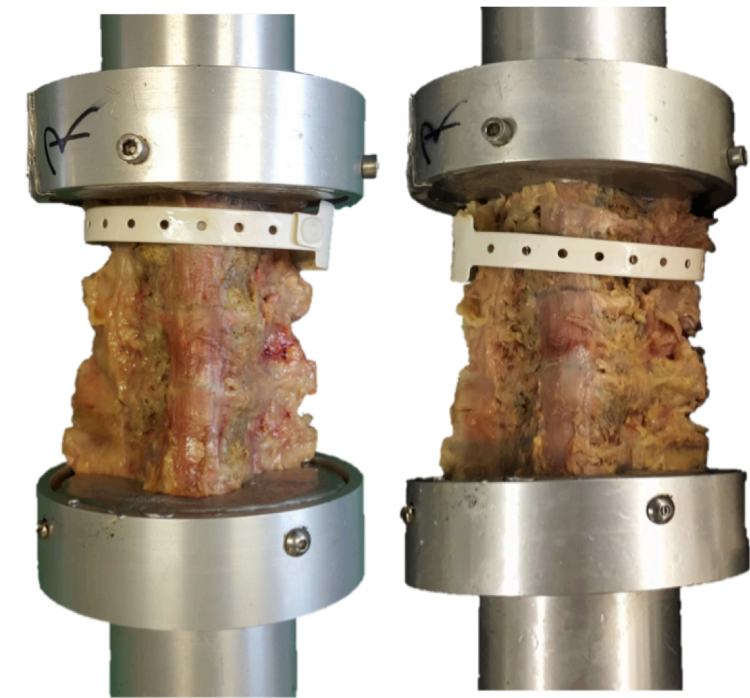


Fig. 2. Fresh spinal specimen before (left) and after embalming with F4L (right).

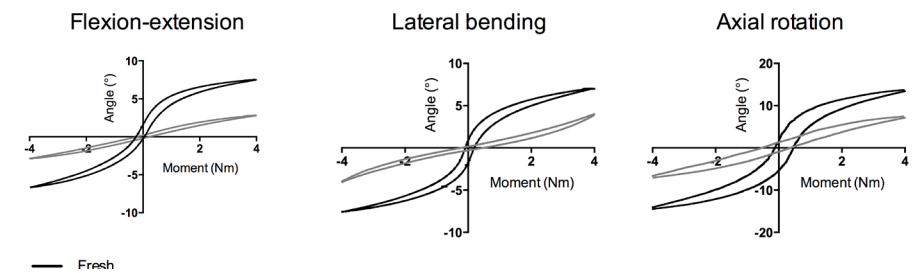


Fig. 3. Typical example of a load-deformation curve in all three loading planes of a single thoracic spine under a load of -4 N m to $+4\text{ N m}$ before (fresh) and after embalming with F4L.

Discussion

Fresh frozen spinal specimens are limited in their use for *in vitro* biomechanical testing due to the risk of infection and short duration of usage due to autolysis. Specimens embalmed with formaldehyde do not suffer from these shortcomings. However, previous work showed that formaldehyde embalming has detrimental effects on the biomechanical properties of the spine [2]. As such, there is an obvious need for novel embalming methods that preserve tissue characteristics. Fix for Life (F4L) is a new embalming method that combines a lower formaldehyde concentration and components that aim to preserve tissue flexibility [3]. The present study analyzed the effects of F4L embalming on spinal biomechanics.

The results demonstrate a significant alteration of spinal biomechanics after F4L embalming. Large increases in stiffness of at least 230% were observed. Moreover, ROM decreased by 46% in FE, 56% in LB and 54% in AR (Fig. 3 and Table 1). While this is clearly less than the reduction of around 80% in all loading directions after formaldehyde embalming reported before [2] it is still too much to allow for adequate testing of implant and surgery effects. Moreover, the effect of F4L embalming seems to be larger than the effect of multiple freeze-thaw cycles, a technique which can be used for long lasting experiments. One study reported increases of ROM in FE by 29%, in LB by 79% and in AR by 42% after three freeze-thaw cycles of human spines [7]. Another study could not identify any significant effects of three freeze-thaw cycles on the spinal biomechanics of porcine lumbar spines [8].

Besides the direct effects of the chemical contents of the F4L embalming method on human tissue, the immersion of the specimens in the various fluids for several weeks has possibly altered tissue hydration. Especially the intervertebral disc is known for its swelling capacity. To limit the effects of altered tissue hydration, a preload was applied for one hour to simulate physiologic upright loading conditions and to limit the effect of overhydrated intervertebral discs [9,10].

No previous biomechanical analysis of human cadaveric spinal specimens embalmed with this new fixation method has been performed. Besides F4L, there have been reports of other embalming methods that aim to maintain physiologic tissue characteristics. Thiel-fixation has been shown to preserve the non-linear load-deformation-characteristics on lumbar spinal segments

from 16 week old calves [11]. However, the ROM increased by 22% in flexion-extension, 23% in lateral bending and 45% in axial rotation [11]. To our knowledge, this method has not been studied with human spines. Another method, AnubiFiX™, has been studied in a cadaveric model for laparoscopic colorectal surgery training [12], but no biomechanical evaluation on spines was performed.

In conclusion, based on this study, F4L does not maintain physiological spinal biomechanical properties and should therefore not be used for *in vitro* studies that require physiological biomechanical properties. Nevertheless, the method may be an alternative to formaldehyde fixation in situations such as training and education because the effect on spinal biomechanics is less detrimental than formaldehyde and tissue color is maintained.

Conflict of interest statement

The authors declare there are no conflicts of interest.

Acknowledgments

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