

Investigation of the uncharacterized 'glue' that holds bone together

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INTRODUCTION

Bone is a complex composite structure consisting of ceramic-like biological apatite and ductile type I collagen. Together, these juxtaposing properties allow for bone to be at once, stiff and tough. Recent literature suggests that the two phases are interpenetrating and that non-collagenous proteins (NCPs) use electrostatic interactions to hold the phases together [1].

This perspective could improve our understanding of bone and lead to novel treatment strategies for fractures and bone fragility. In order to quantify the effect of NCP-bonding on bone properties, hydrated *ex vivo* emu tibial bones were endocortically infused with saline or one of three solutions: 100 ppm, 0.1 M and 1 M NaF in saline.

Negative fluoride ion infusion is hypothesized to neutralize the positive apatite surface charge and repel the negatively charged NCP bridges, leaving both the collagen and mineral intact, but disconnected. This will be tested by comparing the effect of fluoride treatment on the Young's modulus and microhardness of bone.

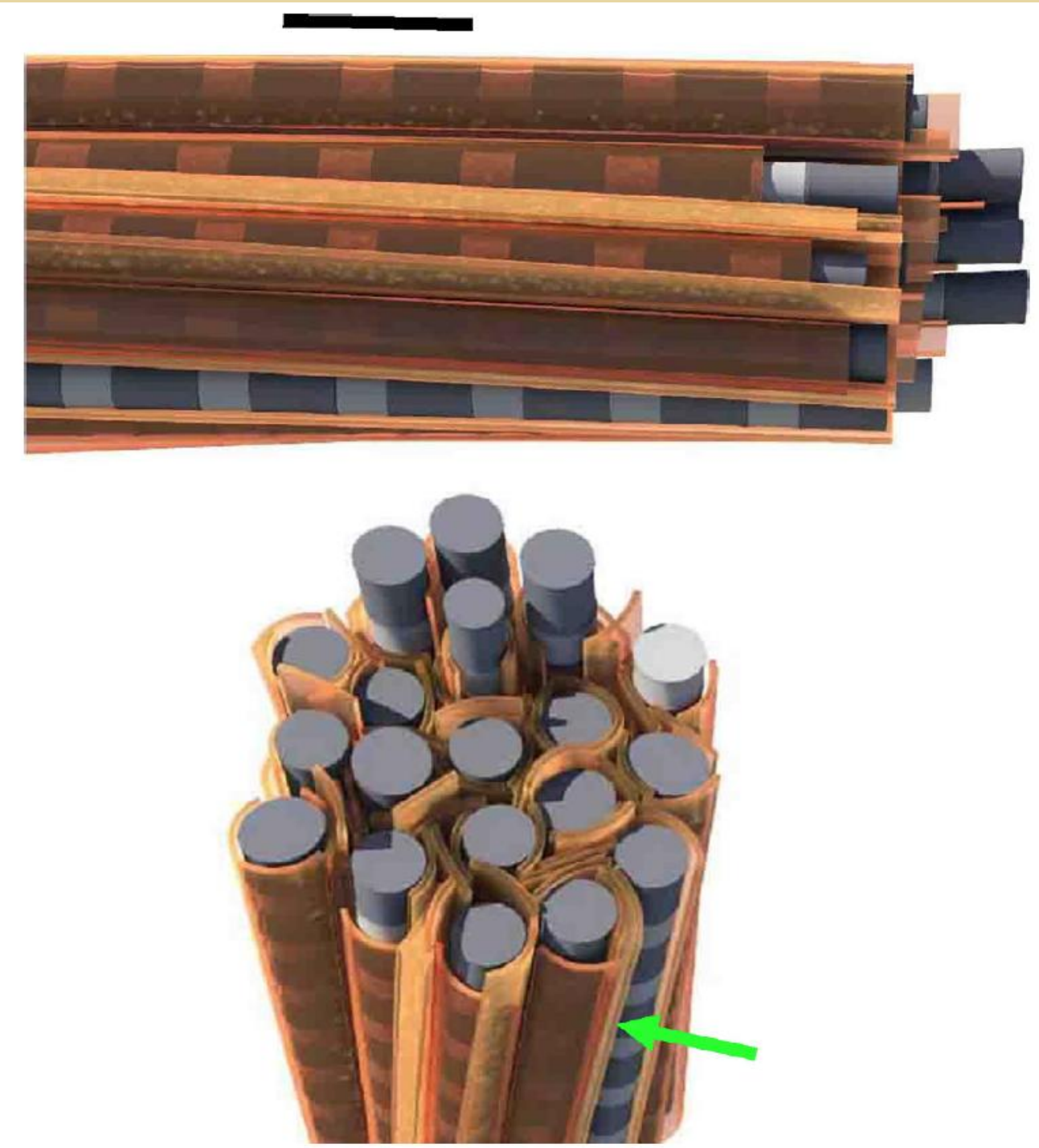


Figure 1: Proposed nanometer scale structure of collagen and mineral in bone. Scale bar represents 100 nm [1]

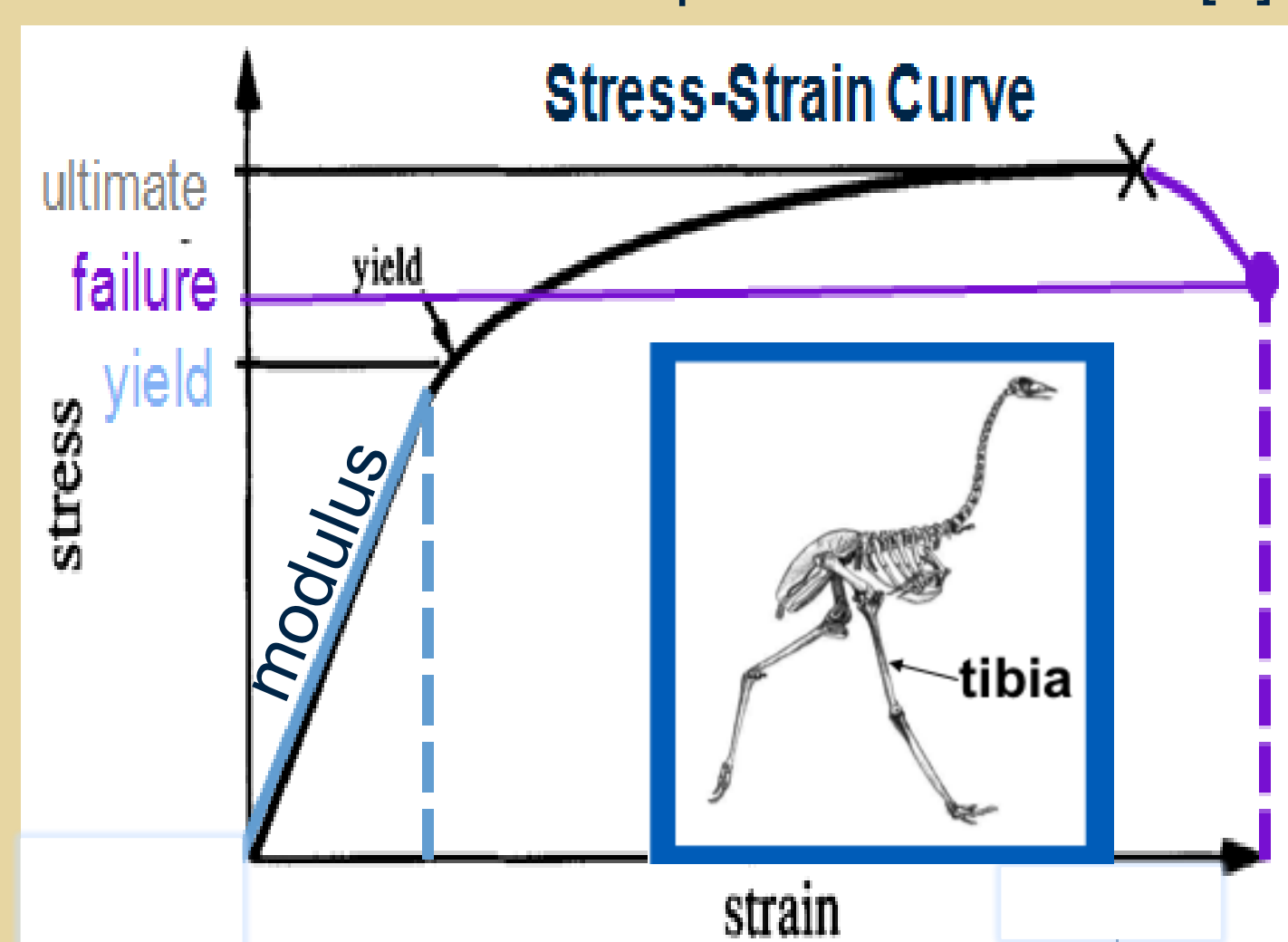


Figure 2: Example stress-strain curve for a tough material [3]

RESULTS

Data were plotted with a Box plot and a Shapiro-Wilk normality test was undertaken to determine if the data were normally distributed.

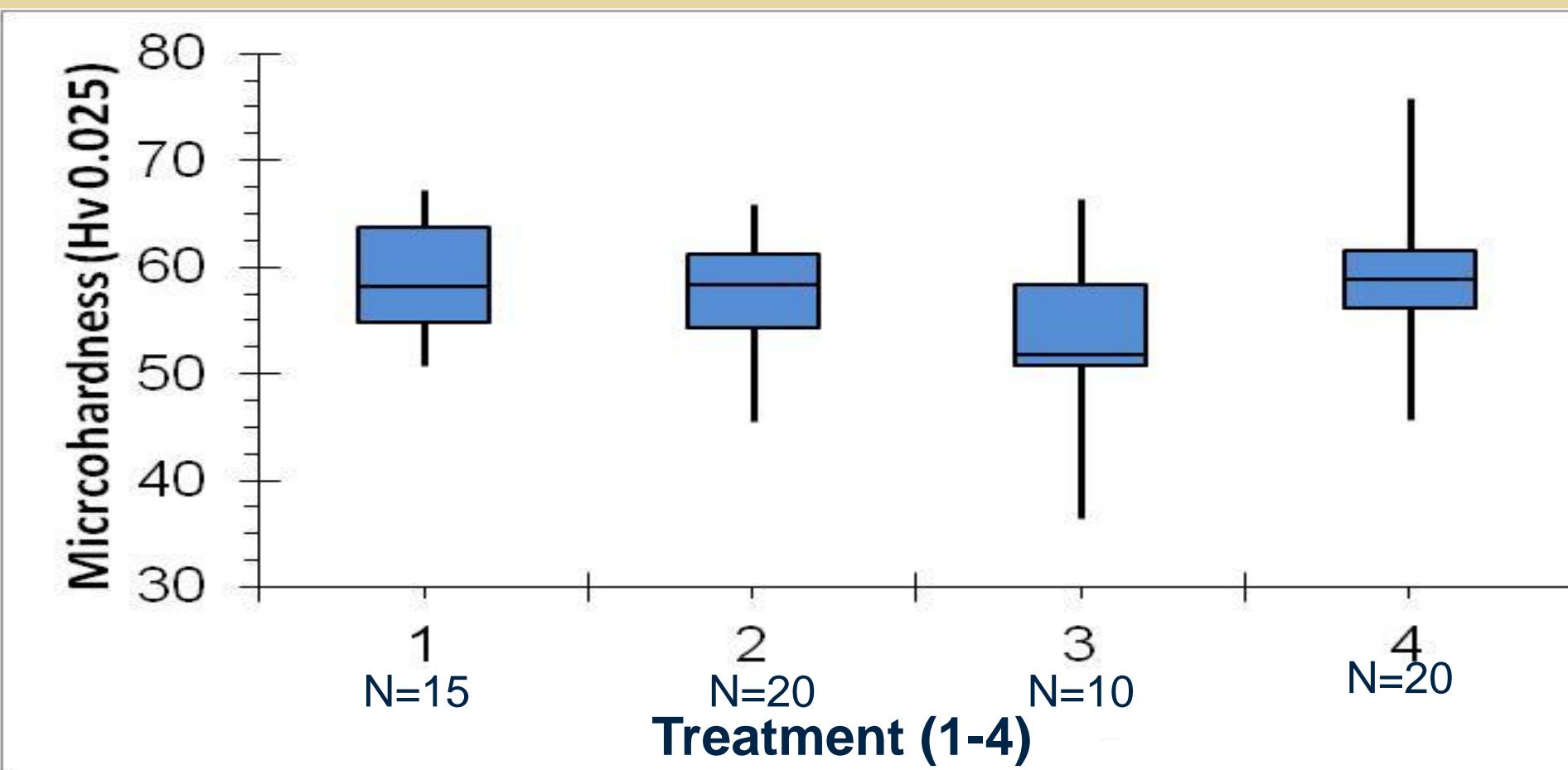


Figure 6: Box plot of microhardness values for treatment type

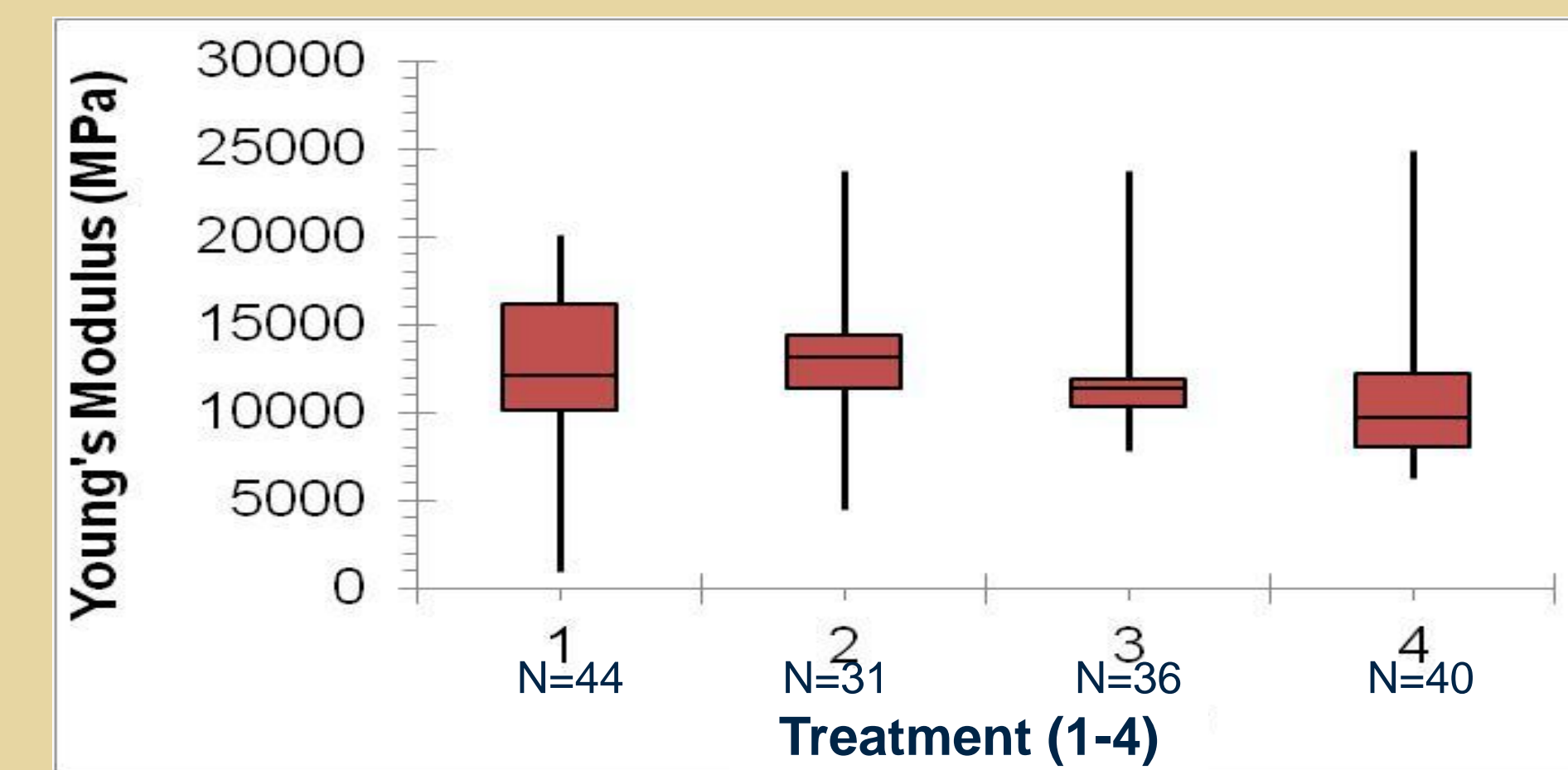


Figure 7: Box plot of Young's modulus values for treatment type

Table 1: Shapiro-Wilk normality test for microhardness of saline and fluoride treated samples

Treatment	p-value
Saline (1)	0.27
100 PPM NaF	0.66
0.1M NaF	0.32
1 M NaF	0.47

The hardness data were normally distributed for all groups.

Table 2: Shapiro-Wilk normality test for Young's Modulus of saline and fluoride treated samples

Treatment	p-value
Saline (1)	0.08
100 PPM NaF	0.37
0.1M NaF	1.6E-6
1 M NaF	3.1E-6

The Young's modulus data for the saline and 100 ppm NaF groups were normally distributed, but the 0.1 and 1 M NaF infusion groups were not.

Emu bone data were plotted along with the theoretical relationship proposed by Evans *et al.* [3]. The saline bones follow the trend direction although they are off-set (Fig. 9). The Vickers hardness of the NaF-treated bones does not change with Young's modulus (Fig. 10).

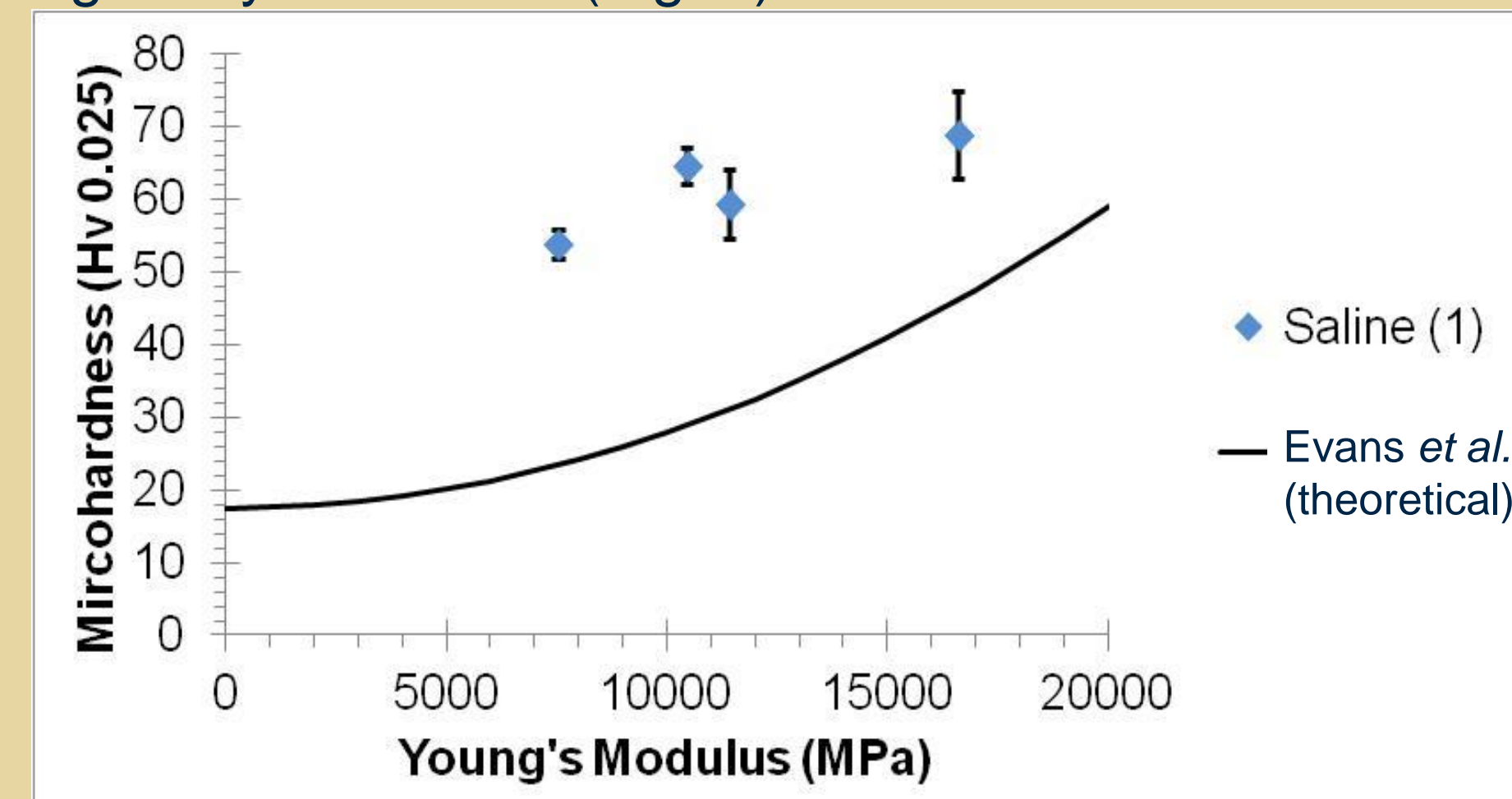


Figure 8: Young's modulus vs microhardness for saline treated bones

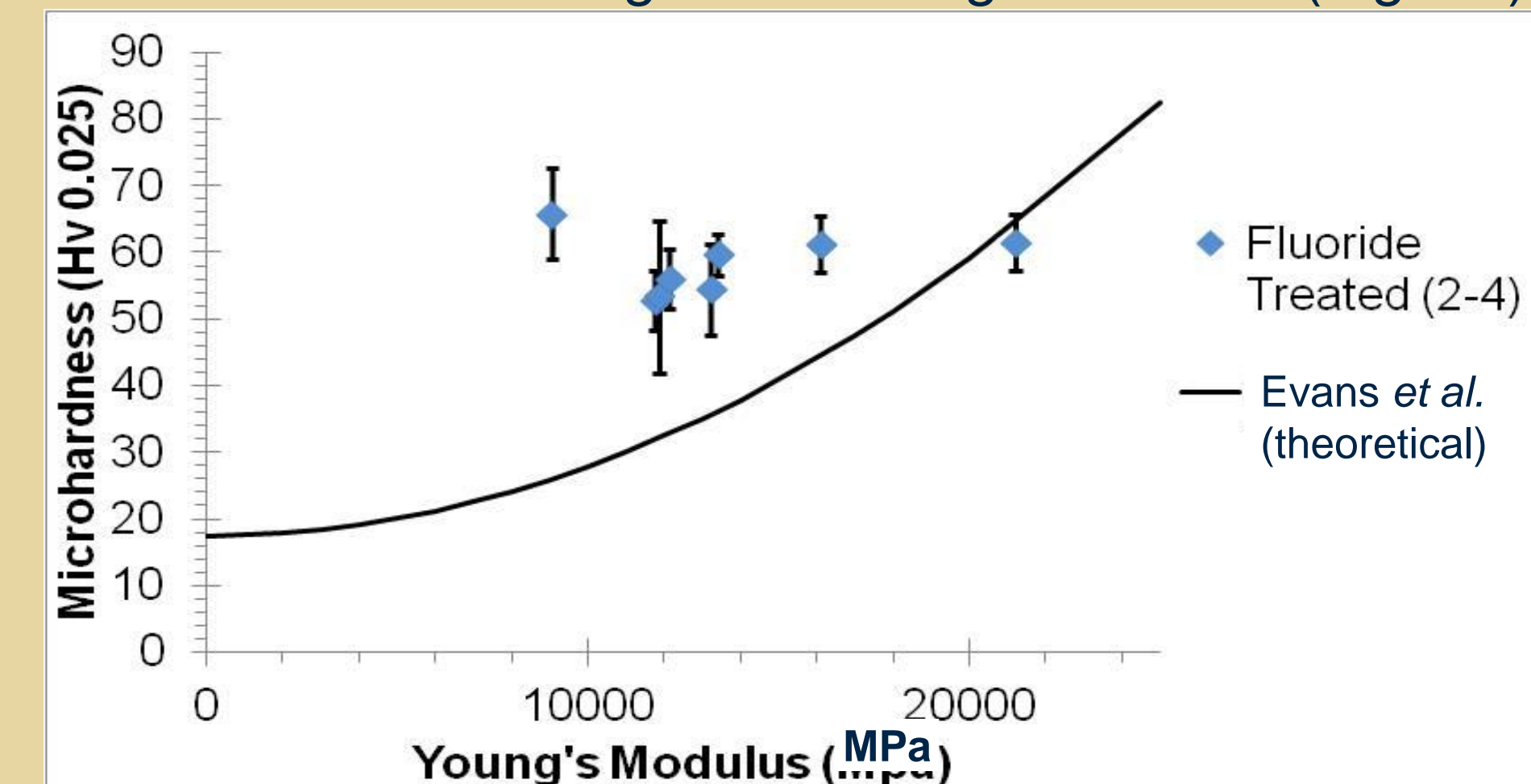


Figure 9: Young's modulus vs. microhardness for NaF-treated bones

PREVIOUS WORK

Emu bone beams (1 mm x 2mm x 20 mm) were mechanically tested in 4-point bending to failure. The Young's modulus for each sample was calculated from the stress-strain values which were calculated from the force-displacement data acquired with the Instron mechanical testing machine. The Young's modulus is the ratio of the stress and strain for the initial linear performance where the material deforms elastically (Fig. 2). A larger Young's modulus indicates a greater material stiffness and an ability to undertake a large amount of stress with comparatively low strain. When the stress is removed in the elastic region, the bone beam returns to its original state. The Young's modulus obtained for 4-pt bending samples from a previous study are used in this study to compare them with the new microhardness data.

METHODOLOGY

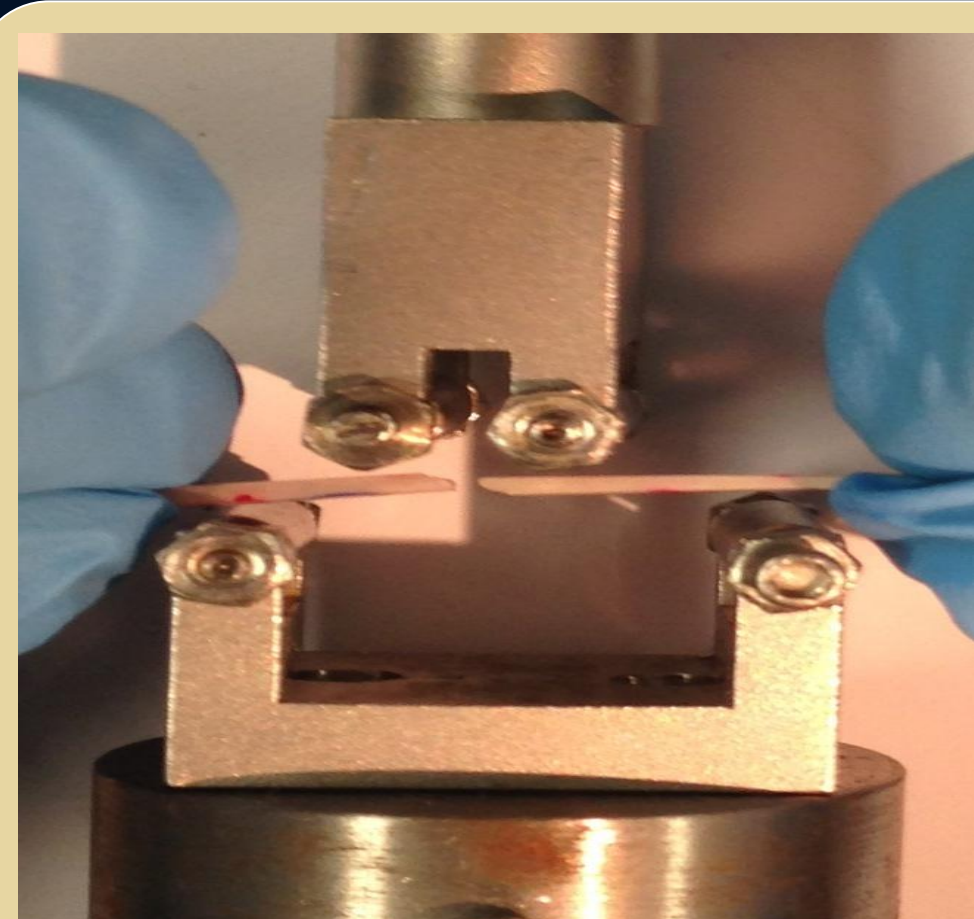


Figure 3: 4-pt bending

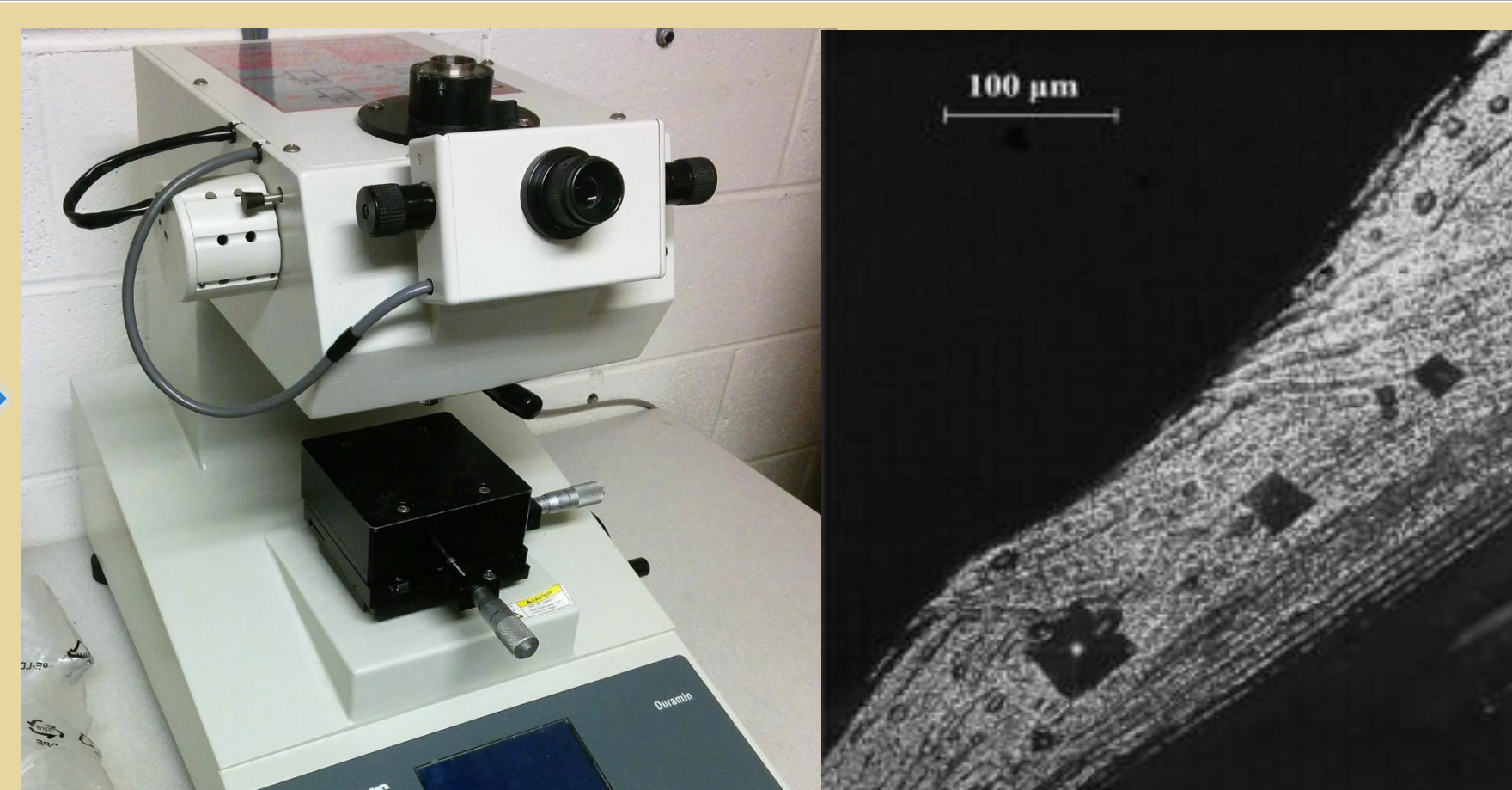


Figure 4: Microhardness Tester, example indent [4], and microhardness measurement process.

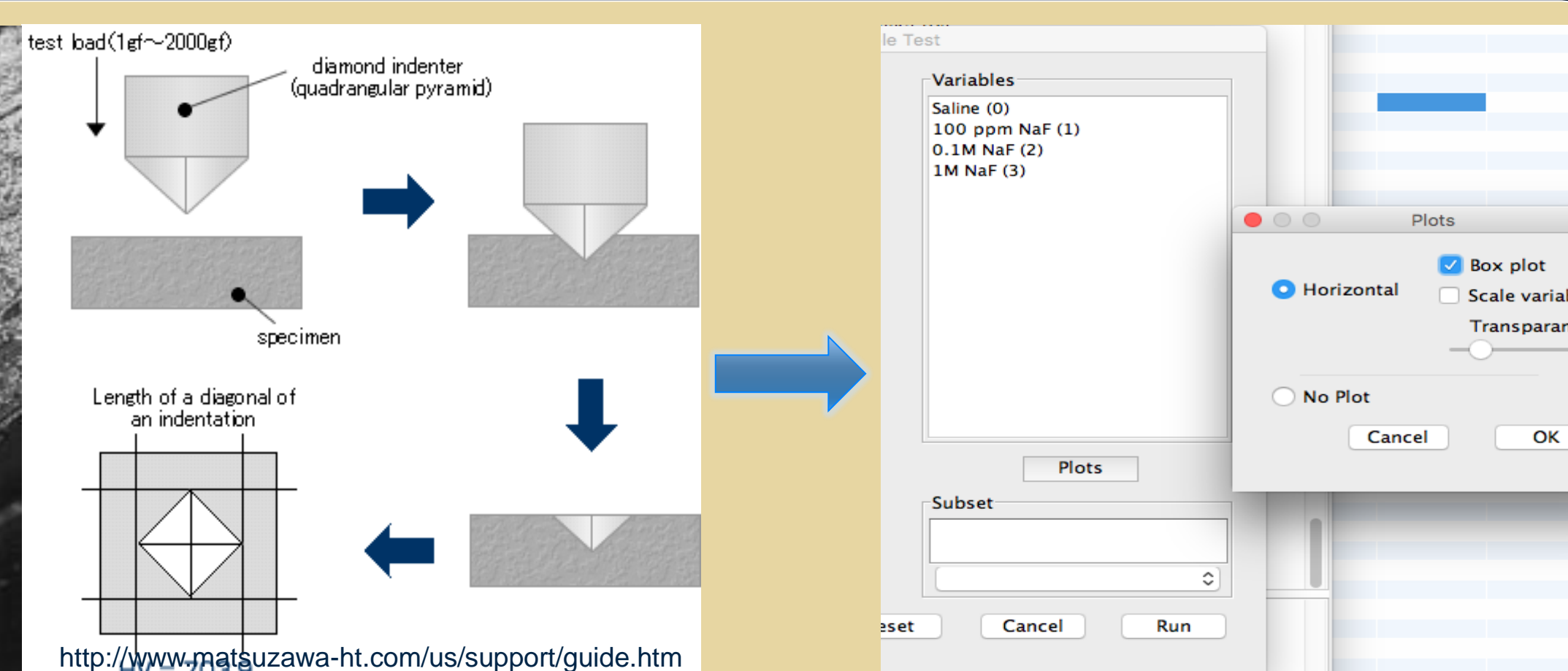


Figure 5: R statistical analysis

HYPOTHESIS

It was hypothesized that the Young's modulus of cortical bone is related to the bone microhardness and mineral volume fractions [3]. This work also suggested that bone performance may require a bond between the mineral and collagen phases, as a synthetic bone analogue composite material with similar mineral fraction had inferior material properties. We assumed that the bone mineral content of the emu cortical bone was similar for all samples, and tested the effect of reducing the mineral-collagen bond strength by infusing the bone with NaF-solutions (0 (control), 100 ppm, 0.1 M and 1 M NaF in saline).

DISCUSSION

The non-Gaussian distribution of the 0.1 M and 1 M NaF-infused Young's modulus (E) values suggests that these values cannot be included in an ANOVA test. This result also suggests that the 0.1 M and 1 M NaF-infused bones may follow different statistics, perhaps Weibull statistics of ceramic materials. The 0.1 and 1 M F may have debonded the ceramic mineral from the ductile collagen. The microhardness and the Young's modulus data for saline bones follow the relationship suggested by Evans *et al.* [3] with an offset. The harder values could be caused by the dry state of the bones. The NaF-treated bones do not follow the same trend, as microhardness did not increase with E. This could be related to the change in statistics observed for 0.1 and 1 M F infused bones.

CONCLUSIONS & OUTLOOK

The Young's modulus (E) of cortical bone infused with 0 and 100 ppm NaF-treated bones and microhardness values are normally distributed, but the E values for 0.1 and 1 M NaF are not. This suggests that the mineral may have been debonded from the collagen with 0.1 and 1 M NaF. The saline data fits a previously reported trend between bone microhardness and E (with an offset), but the hardness of the NaF-treated bones did not correlate the same way with E. We will further pursue the possibility that 0.1 and 1 M NaF-infused bones behave more as a ceramic than a composite material.

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This study consisted of embedding the broken samples in a plastic resin, cutting a flat section, and polishing the bone surface using grit and diamond paste. Once a smooth surface was achieved, Vickers microhardness testing was undertaken. The test was performed by applying 0.25 N of force through a diamond (Vickers) indenter for a duration of 15 seconds. The two diameters of the indent were measured, and the Vickers hardness (HV) calculated with equation 1. 5 values were obtained for each sample.

$$HV \approx 0.1891 \frac{F}{a^2} \left[\frac{kgf}{mm^2} \right] \quad (Eq.1)$$

In order to compare the Young's modulus and hardness data, statistical analysis using R was undertaken. A Gaussian distribution is used in natural sciences to estimate the variation in measurements around a true value. This assumption must be validated as a first step before the statistical analysis of the hardness data. If the data are normally distributed in each group, a one-way ANOVA test can determine if Young's modulus and/or microhardness values were statistically different for the 0, 100 ppm, 0.1 m and 1 M NaF concentrations in saline.