

# STRESS RELAXATION OF DECALCIFIED BOVINE CORTICAL BONE IS FLOW INDEPENDENT

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## INTRODUCTION

Creep and stress relaxation in biological materials are modeled as fluid flow independent (Fung, 1993), fluid flow dependent (Mow et al, 1980) or as a combination of the two (Setton et al, 1993). While each approach is theoretically distinct it can be difficult in practice to determine which is more appropriate for a particular tissue. For decalcified cortical bone our experimental data support the conclusion that stress relaxation is fluid flow independent.

## THEORETICAL METHODS

$$\sigma(t) = \sigma_{eq} + \Delta\sigma_0\phi(t)$$

An equation often used to model uniaxial stress relaxation is:

where,  $\sigma_{eq}$  is the equilibrium stress,  $\Delta\sigma_0$  is the difference between the stress at time zero and at equilibrium and  $\phi(t)$  is the relaxation function that describes the time dependence of stress,  $\sigma(t)$ .

The uniaxial equilibrium (aggregate) modulus  $H_A$  of a material is defined as the ratio of the equilibrium stress to the equilibrium strain of the material. A signature feature of the well known biphasic (flow dependent) theory of poroelasticity is that the characteristic time constant  $T$  of the relaxation function  $\phi(t)$  is proportional to the ratio of the drag force of fluid flowing through the matrix to the aggregate modulus,  $H_A$  (Mow et al, 1980).

Consequently, differences in the aggregate modulus  $H_A$  between specimens result in differences in the characteristic time  $T$  of the stress relaxation process. (For uniaxial confined compression  $T$  is proportional to  $1/(H_A k)$ ,  $k$  is the tissue permeability.)

We exploited the characteristic dependence of  $T$  on  $H_A$  in the biphasic theory by incubating bone specimens in ribose solution to increase the crosslinking in the specimen and, consequently, increase the equilibrium modulus of the collagenous matrix. If stress relaxation in this material is governed by fluid flow the relaxation time constant will vary with  $H_A$ .

## EXPERIMENTAL METHODS

Five mm diameter cylindrical specimens of bovine cortical bone were subjected to *in vitro* ribosylation by incubating in 100 mg/ml of ribose in Hanks buffer/ 1.3 mM  $\text{CaCl}_2$  at 37°C with 100  $\mu\text{l}$ /10 ml of toluene and chloroform and 5 mg/10 ml of gentamicin to prevent bacterial growth. The specimens were demineralized in formic acid sodium citrate solution (1M sodium citrate in 45% formic acid) for a period of eight weeks. Fifteen specimens (6 control and 9 ribosylated for 3, 8, 11, 17, 29 and 38 days) were used for unconfined stress relaxation compression tests. Each demineralized bone cylinder was loaded to a strain of 50% followed by a hold period under strain control. Stress was measured as a function of time and equilibrium modulus calculated as the ratio of equilibrium stress and strain.

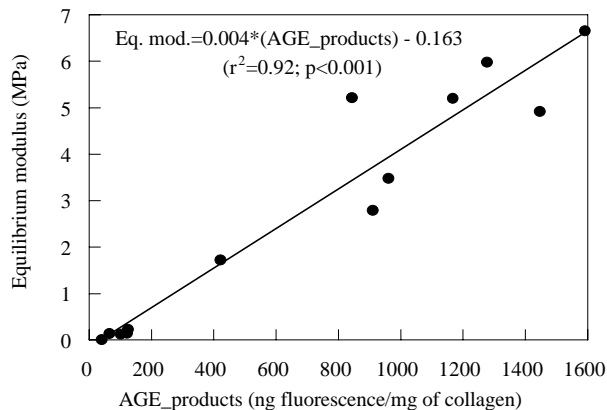
The specimens were papain digested (0.4 mg/ml papain in 0.1M sodium acetate buffer, pH 6.0, 16hrs, 65°C). Ribosylation (AGE products) was determined from fluorescence using 370nm excitation and 440nm emission wavelength standardized against a quinine sulphate solution (Knott et al, 1995). Collagen was determined from hydroxyproline content (Stegemann and Stalder, 1967).

## RESULTS

The time dependence of stress was well fit by the equation,

$$\sigma(t) = \sigma_{eq} + \Delta\sigma_0 e^{-\sqrt{t}/\tau}$$

with  $\tau$  the characteristic time of relaxation, analogous to  $T$  of the biphasic theory. (Adjusted  $r^2$  was  $>0.99$  for eleven specimens and was 0.97 and 0.66 for two).



Statistical analyses (t-tests) of the data indicated that coefficients  $\sigma_{eq}$ ,  $\Delta\sigma_0$  and  $H_A$  were significantly different ( $p < 0.0001$ ) between the ribosylated and control specimens while coefficient ' $\tau$ ' was not ( $p = 0.20$ ). These results were unchanged after adjustment for incubation time. Equilibrium modulus was strongly dependent upon the extent of ribosylation (Figure), however, the

time constant  $\tau$  was not dependent upon equilibrium modulus ( $1/\tau = 0.0011H_A$ ,  $r^2 = -0.42$ ). Note that  $r^2$  negative means the linear statistical model motivated by biphasic theory is worse than assuming  $\tau$  is constant.

## CONCLUSION

Ribosylation of calcified bone significantly increased the equilibrium modulus of the collagenous matrix in direct relationship to the degree of ribosylation. This increase was expected as ribosylation increases the number of crosslinks in the tissue. Against expectation, the characteristic time constant of stress relaxation was not significantly affected by ribosylation. This strongly suggests that the viscous mechanisms of this material are not dependent on equilibrium modulus. As a result, we conclude that fluid flow is not the primary dissipative mechanism in decalcified bone matrix.

Fung YC (1993) *Biomechanics*, Springer-Verlag.

Knott L. et al (1995) *Biochem. J.* 310: 1045-1051.

Mow VC et al, *J Biomech Eng*, 102: 73-84.

Setton L et al (1993), *J Biomech*, 26:581-592.

Stegemann and Stalder (1967) *Clin. Chim. Acta* 18: 267-273.

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