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Comment on, "Effects of exogenous crosslinking on in vitro tensile and compressive moduli of lumbar intervertebral discs" (Clinical Biomechanics 22 (2007) 14–20)

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In their recent publication, Chuang et al examined the effects of genipin-induced collagen crosslinking on the mechanical behavior of lumbar intervertebral discs [1]. Based on their experimental results, Chuang et al concluded, "crosslink augmentation may in fact increase fatigue resistance, and modestly stiffen tissue without a loss of joint range of motion." While this is an interesting result, their conclusions should be reconsidered in light of several caveats: Specifically, this manuscript has mis-characterized some of the experimental results in the cited literature and inappropriately generalized the role of crosslinks in the biomechanical behavior of collagenous tissues.

Crosslinking in collagen can result from a number of different pathways, such as enzymatic maturation by lysyl oxidase [2], non-enzymatic glycation by extracellular sugars [3], or even *in vitro* crosslinking by genipin [4]. These pathways yield molecularly distinct crosslinks, and these crosslinks may contribute differently towards overall tissue biomechanical function.

As a biopolymer, collagen commonly provides structural support for load-bearing tissues. Because the maturation and mechanical competence of collagen is contingent on the formation of enzymatic crosslinks, pathologies can arise when there is inadequate formation of mature crosslinks. The process of collagen crosslinking is initiated by the conversion of telopeptidyl lysine and hydroxylysine residue to aldehyde through the action of lysyl oxidase, a copper metalloenzyme. The hydroxylysine–aldehyde pathway predominates in the telopeptides of bone, cartilage, ligament, tendon, and most major internal connective tissues [5]. Inadequate enzymatic crosslink formation, for example, such as in the abnormal

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inhibition of lysyl oxidase in *Lathyrus odorathus*, results in increased fragility of collagenous tissues [6]. Thus, enzymatic crosslinks play a crucial role in mechanical competence of adult skeletal system. It is important to note, however, the accumulation of enzymatic crosslinks plateaus with skeletal maturity [7], and does not show a direct correlation with the age-related fragility of connective tissues [7, 8].

On the other hand, non-enzymatic glycation (NEG)-induced crosslinks known as Advanced Glycation End-products (AGEs), which occurs spontaneously in the presence of extracellular sugars, are known to accumulate with age in collagenous tissues [7]. The reactive sugars form Schiffs' bases with free amino groups in lysine, hydrosylysine, or arginine residues on the collagen molecule, and the subsequent structure then undergoes Amadori rearrangement that ultimately results in a family of molecules known AGEs [3]. There are numerous studies, including those cited by the authors, which show the deleterious effects of AGEs accumulation on fracture resistance and energy dissipation of the afflicted tissues [8–12].

The authors cited several works, which "lacked direct evidence to support the hypothesis" of "age increasing crosslinks causes tissue brittleness and loss of fatigue resistance in load supporting tissues." Despite their claims, two of the cited manuscripts presented direct experimental evidence of increased crosslinking, mediated by NEG, results in significant tissue stiffening and increased fragility of articular cartilage in both tension [10] and compression [9]. A previous study by Wagner et al specifically tested the effects of crosslinking by NEG on the mechanical performance of the intervertebral disc and found that increased crosslinking significantly correlated with the deterioration of strain energy function and subsequent decreased energy dissipation [13]. There is also a growing body of literature in other collagen-containing tissues such as bone [8,11,12] that show the age-related accumulation of NEG crosslinks are adventitious to the overall mechanical performance of the tissue matrix. In each of the studies cited above, the degree of crosslinking was quantified and inversely related to decreased tissue mechanics. Tissue maturation *in vivo* produces a degree of collagen crosslinking that is optimal for mechanical function, and additional post-translational modifications, such as the age-related accumulation crosslinks caused by non-enzymatic glycation, result in reduced matrix strength.

The study conducted by Chuang et al provides a provocative clinical motivation, but its conclusions may be flawed. Given that the typical bovine animal reaches skeletal maturity at two years, the selection of 4-month old bovine specimens may have resulted in experimentally testing skeletally immature tissue, and the model presented here may not represent an ideal animal model for age-related disc degeneration. In the absence of sufficient enzymatic crosslink accumulation due to incomplete skeletal development, it is thus not surprising that the augmentation of genipin-induced crosslinks enhances the overall tissue mechanics of the intervertebral disc. However, because the crosslinking profiles of the samples were not disclosed, it is not possible to attribute the alterations in tissue mechanics specifically to the type and degree of crosslinking.

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