Importance of perfecting a positive control before testing new hypotheses: the effect of riboflavin and UVA treatment on the corneal stroma of the spiny dogfish shark (*Squalus acanthias*)

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In preparation for studying the effects of treating a variety of marine tissues with riboflavin + long wavelength ultraviolet light (RFUVA), we began by studying the effects of RFUVA on causing tissue crosslinking in strips from dogfish shark corneas, a protocol used successfully during two previous summers at MDIBL. However, during the 2011 summer, no crosslinking was detected as a result of RFUVA, despite varying the concentrations and sources of all known reactants. Thus, the essential "positive control" did not work, for unknown reasons, meaning all the data represent "negative results". It remains unclear why no RFUVA crosslinking occurred.

Keratoconus is a degenerative disease of the cornea, which affects approximately 1 in 2000 people worldwide. Currently, the only treatment approved in the United States for this disease is a corneal transplant. However, a clinical treatment developed in Europe using riboflavin and UVA light (RFUVA) has been found to essentially stop the progression of this degenerative disease by cross-linking molecules of the extracellular matrix of the corneal stroma, thereby strengthening that tissue greatly[5]. Clinical trials of the RFUVA protocol are underway in the U.S. currently. Most of the vertebrate corneal stroma is composed of molecules of Type I collagen[1,3,4], which polymerize to form very fine fibrils arranged in a transparent paracrystalline array of plies that are stacked in an alternating orthogonal pattern parallel to the corneal surface[2]. At the beginning of the summer, a replication of previous studies was performed with RFUVA to assure that the effects on shark tissues could be relied upon to display significant tissue crosslinking, as they had during the summers of 2009 and 2010, thus, a "positive control" to make sure that the entire system worked correctly before proceeding to experimentally determine the effects of this treatment on new, other collagenous tissues, such as the dermis of the spiny dogfish shark (*Squalus acanthias*), or some of the tissues found in the Blue Mussel (*Mytilus edulis*). Result: A positive control using spiny dogfish shark corneas was not observed. That is, there was no significant cross-linking observed in treated versus non-treated strips of shark corneal stroma. Variables such as UVA light intensity, commercial source of shark corneas, commercial source, age, and concentration of riboflavin, pH of solutions, use of a variety of chemical buffers, and source of RO-purified water were all tested without providing any explanation as to what was preventing the cross-linking reaction. Analysis of the MDIBL RO-purified water by mass spectrometry detected no unusual chemicals that might have been absorbing the UVA and thus preventing its absorption by RF, a reaction essential for RFUVA-activated covalent crosslinking to occur. To determine whether the wavelength of the UVA light source changed since the time of its purchase, spectral analysis of the wavelength(s) of light emitted is underway currently.

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