Collagen V2.0: A system for re-engineering human type II collagen

Collagen is the main structural component in animals, making up approximately 30% of the total protein in our bodies. Collagen is an integral component of skin, tendon, bones, cartilage, and the placenta. Modern methods in cell biology have determined a lot of information about collagen: its role in tissues, the types of cells that produce it, the pathways involved in its production, and the sequence of amino acids that make up each molecule of each type of collagen. If the proper sequence of events in collagen production, folding, or secretion into the extracellular matrix is perturbed, even in a subtle manner, serious disorders can result. Mutations in the genes involved in these events cause a wide spectrum of syndromes such as Osteogenesis imperfecta (brittle bone disease), chondrodysplasias (dwarfism), and Ehler-Danlos syndrome (extreme flexibility of skin and limbs). Diet can also affect collagen performance: a lack of vitamin C causes scurvy, characterized by bleeding gums, loss of teeth, discoloration of the skin, and lack of proper wound healing. In addition to the effects of mutation, as we age, collagen production is reduced, and the remaining collagen becomes less flexible.

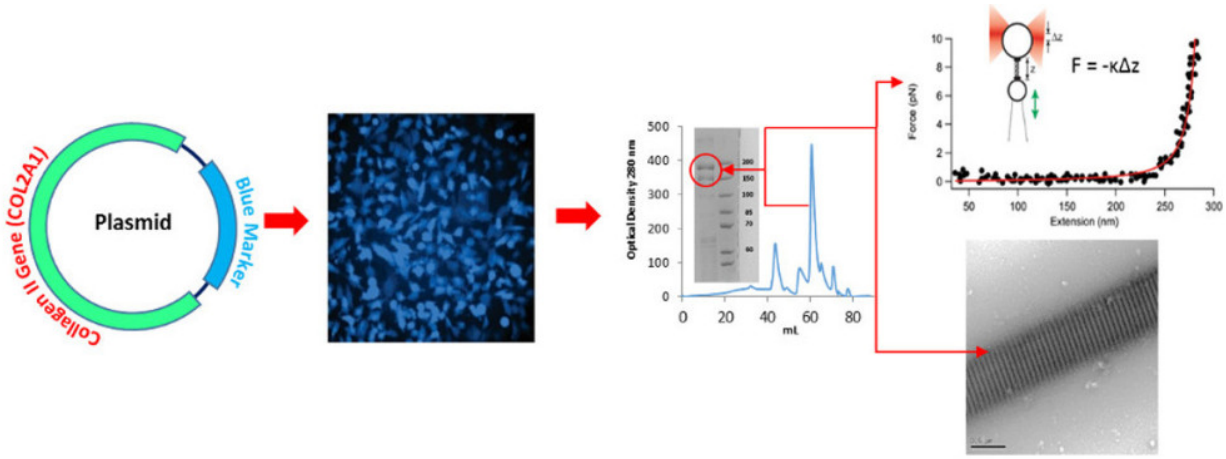

Fig. 1. A plasmid containing the human type II collagen gene with a blue fluorescence indicator of expression is introduced into a cell line capable of secreting collagen. The collagen is purified from the media, then its properties are confirmed by a number of methods, including optical tweezers to determine its single-molecule mechanics and electron microscopy to probe the structure of self-assembled fibrils.

Though many aspects of collagen and its pathologies have been well described, exactly how chemical changes at the molecular level result in pathological physical properties is much less

evident. Questions still remain as to how a single mutation can have such profound effects. Most studies on collagens use protein extracted from animal tissues. Though this approach generally yields large amounts of material, age and diet can influence the quality of the collagen harvested, and, since harvesting results in the destruction of the source, subsequent harvests can have properties that differ from the original, making reliable conclusions difficult.

Our paper reports on a way to overcome this problem. We were able to clone a copy of the COL2A1 gene for human type II collagen into a cell line capable of performing all functions necessary for collagen production. Our paper describes the steps that were taken to insert the gene into a cell line, and the methods used to ensure that protein production would be stable. It then goes on to describe how we obtain the collagen from the cells, and how we purify it from the rest of the proteins present.

The latter part of the paper describes the steps that we took to confirm that our collagen was indistinguishable from that produced in the natural host. Biochemical and biophysical characterization of the secreted, purified protein were used to demonstrate fidelity of the collagen at all levels, starting from confirming that the sequence of amino acids was correct, and then showing that indeed proper post-translational modifications had been performed on the collagen to allow for formation of stable triple helices (the fundamental structural unit of collagen). We then confirmed that triple helices were indeed present, and that they could form fibrils identical to those found in cartilage.

Now that we have shown that it is possible to produce wild-type collagen in all aspects identical to the natural form, this system can be used to introduce specific mutations and to modify nutrients responsible for the diseases described above, to study their effects on how collagen functions. The collagen can also be artificially “aged”. Our system permits tailoring collagen for industrial applications, for the personal care industry, and for investigations into health and disease.

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Publication

Development and characterization of a eukaryotic expression system for human type II procollagen.
Wieczorek A, Rezaei N, Chan CK, Xu C, Panwar P, Brömme D, Merschrod S EF, Forde NR
BMC Biotechnol. 2015 Dec 15