The Science of Elastin

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Elastin is an essential part of various human tissues that depend on elasticity. These connective tissues include the skin, lung and arteries. Elastin provides these elastic tissues with the ability to stretch and recoil and plays a critical role in supporting and maintaining healthy cells (Almine et al., 2010; Daamen et al., 2007; Debelle and Alix, 1999; Keeley et al., 2002; Kielty et al., 2002; Mecham, 1991; Pasquali-Ronchetti and Baccarani-Contri, 1997; Pepe et al., 2007; Rosenbloom et al., 1993; Uitto et al., 1991; Vrhovski and Weiss, 1998). In the skin, most elastin is located in the dermis, which is the springy middle layer (Rosenbloom et al., 1993). This elastic tissue is assembled as a continuous network of fibers that encompasses, with decreasing elastin content, the mature elastic fibers, wispy immature elaunin fibers and oxytalan fibers (Montes, 1996). The dense mass of elastic fibers in the reticular dermis dominates the region and is particularly important to the overall elasticity of the skin. Generally, the most mature, thicker elastin fibers are found deep in the dermis, where they function as an inter-penetrating elastin network (Kielty and Shuttleworth, 1997; Ushiki, 2002).

The protein tropoelastin is the fundamental building component of all elastin. There is only one tropoelastin gene (ELN) in humans, in contrast to many other connective tissue proteins like the collagens that can be members of large, complex gene families (Bashir et al., 1989; Indik et al., 1989; Indik et al., 1987). The expression of this single ELN gene mainly occurs before birth and in the first few years of life when the cells of elastic tissues produce the elastin required for the body to develop. From a young age, ELN expression is turned down substantially as we make less elastin, such that by the time we are middle-aged only a trickle of elastin is produced and we rely mostly on the elastin that was

deposited in the womb and those first few years of life (Cleary et al., 1967; Wirtschafter et al., 1967). The implications of this include the fact that our elastic connective tissues need to rely on the persistence of elastin. To this end, Elastin has been shown to have a half-life of about 74 years (Shapiro et al., 1991) and is the longest lasting protein in the body. However, in the event of damage to the elastin in the skin of adults following, e.g., serious injury such as burns; sun damage; or, simply as a result of aging, the low level of elastin production may mean the damage cannot be efficiently repaired and the skin gradually loses its elasticity.

The primary transcript from the single ELN gene is spliced to give different forms of the tropoelastin protein that either lack or contain various exons, which in turn give rise to forms of tropoelastin that can vary slightly in their protein sequence (Indik et al., 1989; Indik et al., 1987). The implications of this splicing variety are not clear although some exons are always present, while others are occasionally spliced out. For example, exon 26A is unique to humans and appears to be spliced out in healthy elastic skin tissue but is occasionally present under conditions of elastin damage, such as following UV exposure (e.g. sun damaged skin) or extreme temperature treatments (Chen et al., 2009; Schmelzer et al., 2005). As such, it may be that some forms of the tropoelastin protein are associated with healthy elastic tissues, while other forms are associated with injury or disease.

As a key step in making elastin, many tropoelastin molecules associate and are then cross-linked, or connected, to form insoluble elastin (Mithieux and Weiss, 2005; Rosenbloom et al., 1993; Vrhovski and Weiss, 1998). The process of elastic fiber formation is also known to include a number of other molecules. The microfibrils, of which fibrillin-1 is the major component, are structures present in the extracellular matrix which are thought to anchor elastic fiber formation. Cross-linking tropoelastin spherules are introduced to the microfibrils by the molecules fibulin-5 and possibly fibulin-4 (Yamauchi et al., 2010) and accrete on pre-existing elastin. Fibulin-2 may work cooperatively with fibulin-5 to assist in elastic fiber formation (Chapman et al., 2010). Emilin-1 may also regulate oxytalan fiber formation but does not appear to directly regulate elastin

expression or deposition (Nakatomi et al., 2011). The cross-linking of tropoelastin is carried out by lysyl oxidases – a family of five enzymes (LOX and LOXL, LOXL 2-4) which are likely to redundantly contribute to the crosslinking process. LOX knockout mice show a reduction in elastin cross-linking (Maki et al., 2005). In addition, LOX and LOXL have both been detected by immunohistochemistry in the dermis and epidermis of normal human foreskin and dermal equivalents and their expression levels have been shown to decrease with age (Noblesse et al., 2004; Sohm et al., 2010).

Figure 1, below, provides a schematic representation of what is currently understood to be the process by which elastin fibers are formed.

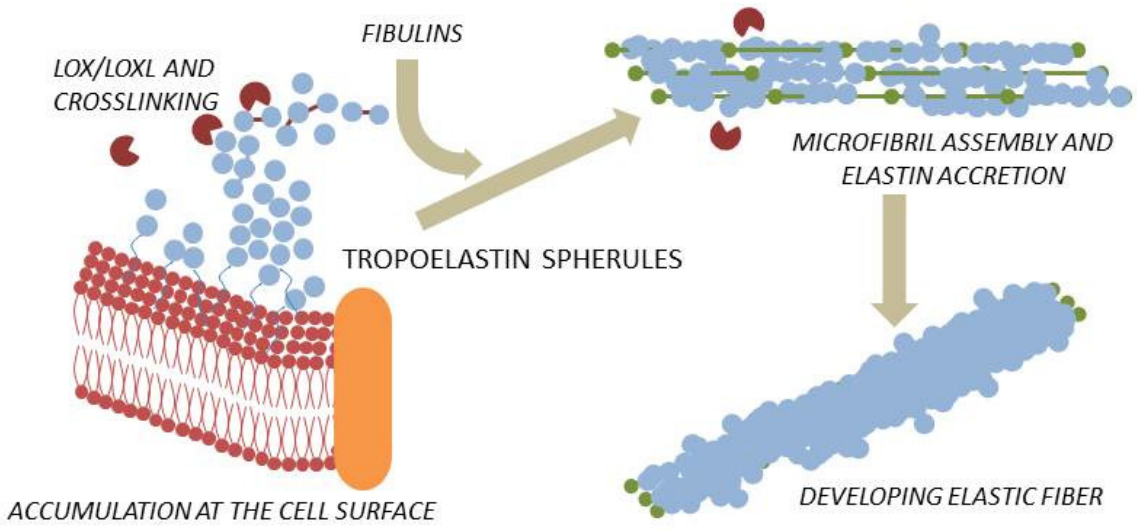

Figure 1: Schematic representation of Elastin fiber formation. Tropoelastin is expressed then secreted as a mature form of the protein into the extracellular matrix. This tropoelastin accumulates on the cell surface, first as small particles then as larger, approximately 1 micron spherules that are effectively massively associated coacervates of tropoelastin. At an undefined stage, the tropoelastin is subjected to oxidation by lysyl oxidase enzymes at a subset of lysines which subsequently participate in aldol condensation and Schiff base reactions to form cross-links. The forming elastin is introduced to microfibrils in the extracellular matrix by members of the fibulin protein family where the elastin fibers are assembled. The resulting elastin is a very stable and persistent structure that has an impressive ability to confer recoil to human tissues (Debelle and Alix, 1999;

Keeley et al., 2002; Muiznieks et al., 2010; Urry, 1988).

Given the importance of elastin to the skin and its loss in the aging process, it is not surprising that various attempts have been made to maintain or replenish elastin levels. Treatments aiming to repair or regenerate elastin in elastic tissues should consider all the molecules implicated in elastin fiber formation. However, as elastin fibers develop, they ultimately consist of over 90% elastin and so the integration of sufficient tropoelastin into elastin fibers is clearly the major target. Effective treatment approaches are also restricted due to the obvious physical challenge of transferring materials and/or treatments across the epidermis and into the dermis, resulting in a preference for small molecules and physical treatments. Tretinoin or all-trans retinoic acid is a small molecule that has been used for many years in topical formulations to increase elastin production in skin through increased tropoelastin (Bergstrom, 2009; Tajima et al., 1997) and fibrillin expression and secretion (Watson et al., 2008). Molecules such as aldosterone and mineralocorticoid receptor antagonists can impact on elastin fiber deposition in skin (Mitts et al., 2010). Soy (Zhao et al., 2009) and rice (USPTO 19469891) extracts may also increase elastin formation, as can a combination of zinc and copper (Mahoney et al., 2009). Hyperthermia can increase tropoelastin expression and elastic fiber deposition (Murphy et al., 2010) although this may encourage tropoelastin that contains sequences encoded by exon 26A, a region associated with abnormal elastin structures (Chen et al., 2009). When delivered by retroviral overexpression, the extracellular matrix component versican V3 increases tropoelastin expression in disease cells (Hinek et al., 2004). More recently, a dill extract has also been shown to have the potential to promote elastin formation by promoting LOXL synthesis and secretion into the dermis (Cenizo et al., 2006; Sohm et al., 2010).

However, the major challenge which all of the above approaches need to overcome is the very low level of expression of tropoelastin in the adult skin, meaning that such treatments are likely to only have incremental benefits on the density of skin elastin (Sephel et al., 1987).

Researchers have for many years pursued elastin based materials which may be

useful in the repair or regeneration of elastic tissues (Antonicelli et al., 2009; Lupo and Cole, 2007; Zhang and Falla, 2009). Such materials have evolved from fragments of elastin isolated from animal tissue through to synthetic proteins which mimic the elastic nature of tropoelastin. However, to date none of these materials have been capable of truly mimicking the properties of elastin or the tropoelastin building block which combine remarkable physical properties with an ability to support and stimulate the growth of cells present in elastic tissues. Tropoelastin is amongst the most elastic of all known natural proteins (Holst et al., 2010), with an ability to stretch eight times its resting molecular length and recoil without damage to the protein. The adaptation of recombinant gene technology to allow the production of large quantities of clinical grade human tropoelastin, identical to that present in normal human skin, suggests a promising next generation of products for the maintenance and repair of elastin levels in the skin and other elastic tissues is now possible.

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