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Elastin Based Constructs

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1. Introduction

Elastin is a key structural protein found in the extracellular matrix (ECM) of all mammals. As the dominant part of the elastic fiber, elastin confers the mechanical properties of resilience and elasticity essential to the function of elastic tissues. Elastin interacts with cells through specific biochemical mechanisms. This chapter considers the (1) mechanical and biochemical roles of elastin in elastic tissues and the subsequent disease phenotypes that result from the degradation and loss of elastin, (2) development and success of current elastin based biomaterials including sources of elastin for tissue engineering and their application, and (3) vascular constructs that our laboratory has developed from recombinant human tropoelastin. These constructs mimic the physical and biochemical properties of native elastin.

2. Elastin formation *in vivo*

Elastin is formed in the process of elastogenesis through the assembly and cross-linking of the protein tropoelastin (Figure 1). The tropoelastin monomer is produced from expression of the elastin gene during perinatal development by elastogenic cells such as smooth muscle cells (SMCs), endothelial cells, fibroblasts and chondroblasts (Uitto, Christiano et al. 1991). The tropoelastin transcript undergoes extensive alternative splicing leading to the removal of entire domains from the protein. In humans, this splicing results in several tropoelastin isoforms, the most common of which lacks exon 26A (Indik, Yeh et al. 1987). Mature, intracellular tropoelastin associates with the elastin binding protein (EBP) and this complex is secreted to the cell surface (Hinek 1995). Competition from galactosides results in the dissociation of EBP from tropoelastin and the return of EBP to the cell (Mecham 1991). Released tropoelastin on the cell surface subsequently aggregates by coacervation. During this process, the hydrophobic domains of tropoelastin associate and tropoelastin molecules become concentrated and increasingly aligned allowing for subsequent formation of cross-links (Vrhovski, Jensen et al. 1997).

Coacervated tropoelastin is deposited onto microfibrils which probably serve as a scaffold to direct tropoelastin cross-linking and consequential elastic fiber formation. Cross-linking is facilitated by the enzyme lysyl oxidase, which deaminates lysine side chains in tropoelastin to form allysine sidechains that can subsequently react with adjacent allysine or lysine side chains to form cross-links (Kagan and Sullivan 1982). These cross-links can then further react to form desmosine and isodesmosine cross-links between tropoelastin molecules

(Umeda, Nakamura et al. 2001). Multiple cross-links result in the mature insoluble elastic fiber.

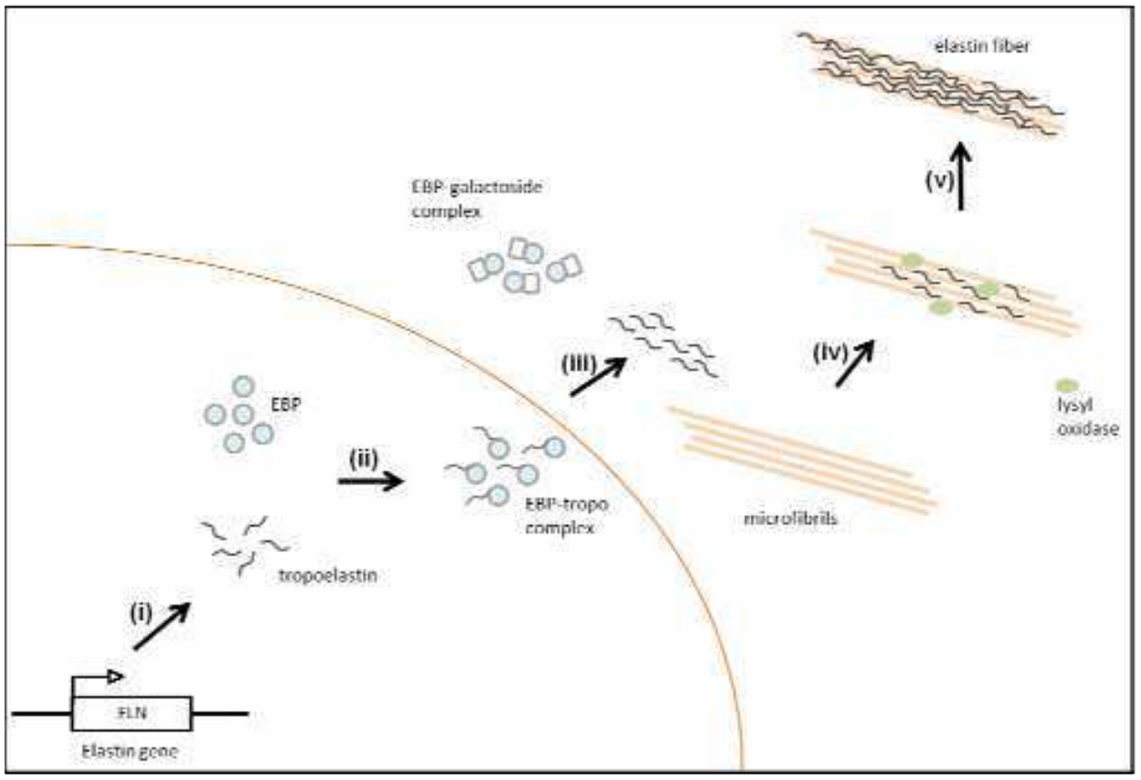

Fig. 1. Schematic of the stages of elastogenesis.
(i) Tropoelastin is transcribed and translated from the elastin (ELN) gene and (ii) transported to the plasma membrane in association with EBP. (iii) Tropoelastin is released and aggregates on the cell surface, while EBP disassociates to form a complex with available galactosides. (iv) Tropoelastin aggregates are oxidized by lysyl oxidase leading to cross-linked elastin that accumulates on microfibrils which help to direct elastin deposition. (v) The process of deposition and cross-linking continues to give rise to mature elastic fibers

3. The role of elastin *in vivo*

Elastin plays a key structural role in elastic tissues including arteries, skin, ligament, cartilage and tendons (Sandberg, Soskel et al. 1981). As the dominant part of the elastic fiber, elastin confers resilience and elasticity essential to the function of these tissues. The arrangement of elastin in the ECM varies between different tissues to yield a wide range of structures with tailored elastic properties. For example, elastin in the form of thin lamina in the arterial wall is mostly responsible for the strength and elasticity necessary for vessel expansion and regulation of blood flow (Glagov, Vito et al. 1992). In the lung, elastin is arranged as a latticework that helps to support the opening and closing of the alveoli (Starcher 2001). In skin, elastin fibers are enriched in the dermis where they impart skin flexibility and extensibility (Roten, Bhat et al. 1996; Pasquali-Ronchetti and Baccarani-Contri 1997).

3.1 Mechanical properties of elastin

Elastin is extremely durable protein with a mean residence time of 74 years (Shapiro, Endicott et al. 1991). It comprises almost 90% of the elastic fiber where it dominates its elastic, mechanical properties. The Young's modulus for elastic fibers typically ranges from 300 - 600 kPa although it can measure as low as 100 kPa for arterial elastin, highlighting the versatile nature of these structures within the ECM (Mithieux and Weiss 2005; Zou and Zhang 2009). Although the mechanism for elasticity has not been fully elucidated, elastic recoil likely to be entropically driven whereby extension of the protein results in a more ordered structure and thus recoil occurs so the protein can return to a disorder state (reviewed by (Rosenbloom, Abrams et al. 1993; Vrhovski and Weiss 1998). This elasticity is due to the inherent elastic properties of the monomer (Holst, Watson et al. 2010; Baldock, Oberhauser et al. 2011).

3.2 Biological properties of elastin

Elastin plays key biological roles in the regulation of cells native to elastic tissues. Studies of elastin knockout mice reveal a crucial role for elastin in arterial morphogenesis through regulation of SMC proliferation and phenotype (Li, Brooke et al. 1998). This model is supported by *in vitro* studies showing that elastin can inhibit SMC proliferation in a dose dependent manner (Ito, Ishimaru et al. 1998). Elastin can also mediate the attachment and proliferation of endothelial cells from several vascular origins (Ito, Ishimaru et al. 1998; Williamson, Shuttleworth et al. 2007; Wilson, Gibson et al. 2010). Similar effects have been observed for dermal fibroblasts (Bax, Rodgers et al. 2009; Rnjak, Wise et al. 2011). Additionally elastin is a chemoattractant for SMCs, endothelial cells and monocytes (Senior, Griffin et al. 1980; Wilson, Gibson et al. 2010).

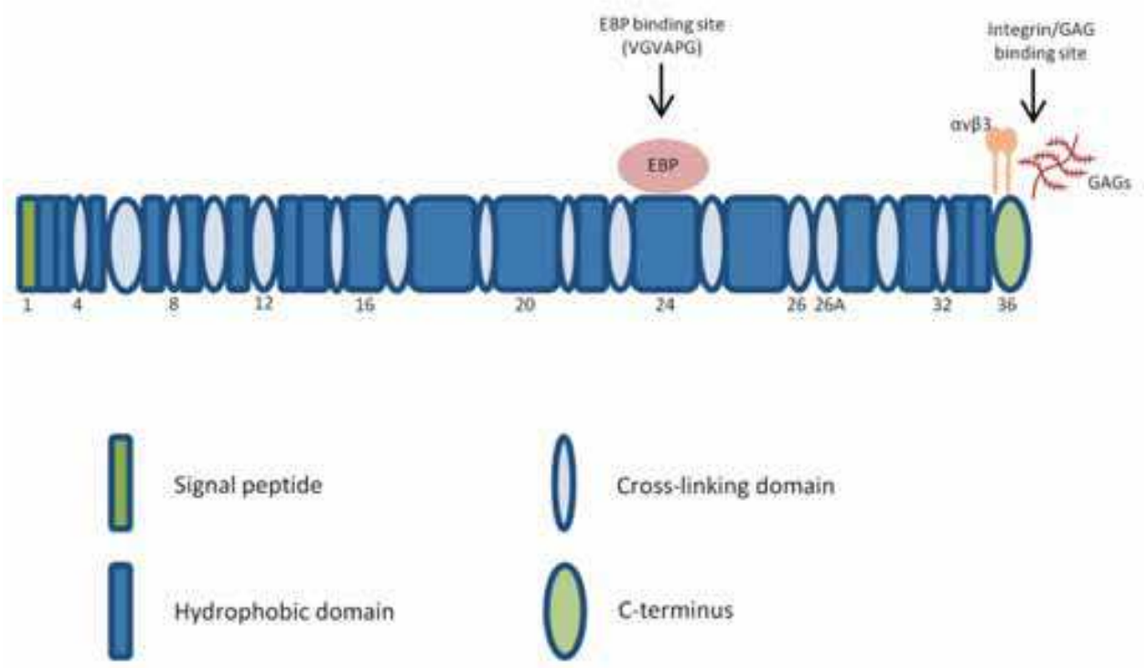

Fig. 2. Schematic of human tropoelastin primary organization and binding partners. All domains are shown. Exons 13, 22, 23, 26A and 32 are subject to alternate splicing

Several cell receptors have been identified for elastin (Figure 2). The most well documented of these receptors is EBP, which binds to multiple sites including the VGVAPG sequence on exon 24 of tropoelastin (Rodgers and Weiss 2005). Upon binding elastin, this receptor activates intracellular signaling pathways involved in cell proliferation, chemotaxis, migration and cell morphology for a range of cell types including SMCs, endothelial cells, fibroblasts, monocytes, leukocytes and mesenchymal cells (Senior, Griffin et al. 1980; Indik, Abrams et al. 1990; Faury, Ristori et al. 1994; Faury, Ristori et al. 1995; Kamisato, Uemura et al. 1997; Jung, Rutka et al. 1998; Mochizuki, Brassart et al. 2002). Other cell receptors, including a less documented glycoprotein termed elastonectin and G protein-coupled receptor can bind elastin through the VGVAPG sequence (Hornebeck, Tixier et al. 1986). Interactions of vascular cells with elastin via these receptors have been shown to dictate focal adhesion formation, cell proliferation and migration (Hornebeck, Tixier et al. 1986; Karnik, Brooke et al. 2003; Karnik, Wythe et al. 2003). Glycosaminoglycans on the SMC and chondrocyte cell surface dominate binding to the C-terminus of bovine tropoelastin (Broekelmann, Kozel et al. 2005; Akhtar, Broekelmann et al. 2011). Cell interactions with human tropoelastin C-terminus specifically occur through the integrin $\alpha_v\beta_3$ (Rodgers and Weiss 2004; Bax, Rodgers et al. 2009). Elastin binding for some cell types is likely to occur through multiple receptors (Bax, Rodgers et al. 2009; Wilson, Gibson et al. 2010; Akhtar, Broekelmann et al. 2011).

4. Elastin and disease

Disease phenotypes manifest due to the degradation and loss of elastin through injury, genetic mutation or age. For example, autosomal dominant and recessive forms of cutis laxa mutations can arise from genetic modifications to the elastin gene and impaired vesicular trafficking, and have been reviewed elsewhere (e.g. (Hucthagowder, Morava et al. 2009; Callewaert, Renard et al. 2011). In skin, the loss of elastin in the dermal layers in severe burns leads significant physical injuries including scarring, wound contraction and loss of skin extensibility (Rnjak, Wise et al. 2011). In the vasculature, genetic mutations in the elastin gene or genes associated with elastic fiber formation result in severe, debilitating diseases (reviewed by (Kielty 2006)). Supravalvular aortic stenosis can arise from point mutations, deletions or translocations within the elastin gene that typically lead to haploinsufficiency and an altered organization of elastic lamellae in the artery, SMC hyperproliferation, increased media thickness and obstruction of the aorta (Urban, Zhang et al. 2001). Elastin is also associated with several vascular pathologies. Damage and fragmentation of elastin in the artery have been linked with deregulation of SMC phenotype, SMC hyperproliferation and invasion which cause vessel occlusion and cardiovascular complications (Brooke, Bayes-Genis et al. 2003). The failure of inelastic materials as arterial replacements further indicate the essential need for intact elastin in functional arteries (Abbott, Megerman et al. 1987).

5. Elastin biomaterials

Common to all elastin diseases is the catalogued *in vivo* inability to adequately regenerate and repair dysfunctional elastic fibers leading to subsequent failure of tissue function. This deficiency is mostly attributed to exclusive expression of elastin during early development

(Mecham 1991), which results in poor renewal of elastin in adult tissues. Materials that can serve as elastin replacements in adult tissues are in demand. This demand is most apparent in vascular tissue engineering as cardiovascular disease is the major contributor to adult mortality worldwide (Lloyd-Jones, Adams et al. 2010). Current synthetic vascular biomaterials, particularly expanded polyterafluoroethylene (ePTFE) and polyethylene terephthalate (Dacron) are poorly mismatched to native arteries in terms of mechanical properties, endothelial cell and SMC interactions and thrombogenecity which lead to a high failure rate in patients (Chlupac, Filova et al. 2009). Elastin can restore properties that are deficient in current grafts, including compliance and strength to match native vessels and regulation of endothelial and smooth muscle cells.

6. Decellularized tissues as elastin biomaterials

Decellularized tissues, generated by the removal of the cellular components of tissue explant are useful as biomaterials as they *a priori* possess much of the complex architecture of the native ECM. Elastic tissues are particular amenable to this method as the stability and insolubility of the elastin protein means it is resistant to many treatments used during decellularization processes.

Decellularizing elastin-rich tissues have been proposed as a path towards the potential replacement of artery, heart valves, bladder skin and lung (Daamen, Veerkamp et al. 2007; Petersen, Calle et al. 2010; Price, England et al. 2010). Enriched elastin vascular grafts generated by decellularization and removal of collagen with proteases from porcine carotid arteries can support fibroblasts *in vitro* (Chuang, Stabler et al. 2009). Cell infiltration has also been observed for other decellularized vascular constructs *in vitro* and *in vivo* (Schmidt and Baier 2000; Conklin, Richter et al. 2002; Dahl, Koh et al. 2003; Uchimura, Sawa et al. 2003). Skin replacements formed from decellularized porcine dermis containing 30% elastin show vascularization and support of cultured keratinocytes when examined in a rat excision model. Degradation of the collagen component of the material also occurs (Hafemann, Ensslen et al. 1999). Transplant of a repopulated decellularized human trachea demonstrates the feasibility of acquiring functionality and improved mechanical capabilities in a patient after 4 months (Macchiarini, Jungebluth et al. 2008).

Despite these advantages, decellularized tissue sources are generally animal derived and are therefore restricted in shape, size and supply. Additionally, decellularization methods involve chemical, physical or enzymatic treatments that can individually or collectively compromise mechanical and biological properties (Gilbert, Sellaro et al. 2006). The common use of detergents can limit the degree of cell repopulation. Decellularization methods are highly specific to a particular tissue thus their broader application to different tissues yields viable results in terms of remaining ECM structure and degree of decellularization (Gilbert, Sellaro et al. 2006). Lack of uniformity and versatility can limit the use of decellularized materials as commercial tissue replacements.

7. Tissue derived elastin constructs

7.1 Insoluble elastin materials

Elastin used for *in vitro* work is generally obtained by purifying the protein directly from elastin-rich tissues. Tissues are treated with chemicals such as NaOH or guanidine-HCl and/or high heat to remove other proteins and cellular material and leave insoluble elastic

fibers. However extensive cross-linking and the consequential insolubility of elastin makes it difficult to manipulate *in vitro* (Daamen, Veerkamp et al. 2007).

Freeze-dried scaffolds of insoluble elastin fibers and purified collagen fibers present mechanical properties consistent with those of elastic tissues (Buttafoco, Engbers-Buijtenhuijs et al. 2006). Furthermore, these scaffolds appear to be compatible with SMCs (Buijtenhuijs, Buttafoco et al. 2004; Engbers-Buijtenhuijs, Buttafoco et al. 2005; Buttafoco, Engbers-Buijtenhuijs et al. 2006), endothelial cells (Wissink, van Luyn et al. 2000) and platelets (Koens, Faraj et al. 2010) pointing to potential vascular applications. Also, insoluble elastin/collagen scaffolds have been explored as possible dermal replacements as these materials can support fibroblasts (Daamen, van Moerkerk et al. 2003) and keratinocytes (Lammers, Tjabringa et al. 2009). Other insoluble elastin composites such as elastin/fibrin biomaterials have been generated but characterization of these materials is limited to mechanical capacity (Barbie, Angibaud et al. 1989).

7.2 Hydrolyzed elastin materials

The solubility of tissue-derived elastin can be improved by partial hydrolysis. A fragmented elastin preparation termed α-elastin is obtained by hydrolysis with oxalic acid and is often used in *in vitro* studies of elastin (Partridge, Davis et al. 1955). Hydrolysis can be performed with potassium hydroxide to yield κ-elastin or through mild digestion with proteinases (Partridge, Davis et al. 1955; Jacob and Hornebeck 1985). Hydrolyzed preparations of elastin display various properties that are similar to the native protein including temperature-induced aggregation (coacervation) and regulation of SMC and fibroblast phenotype (De Vries, Zeegelaar et al. 1995; Ito, Ishimaru et al. 1998). Fragmentation of elastin is associated with reduced protein structural integrity and altered cellular signaling properties (Daamen, Veerkamp et al. 2007; Bax, Rodgers et al. 2009).

Multiple vascular materials have been synthesized from hydrolyzed elastin preparations (Table 1). Hydrogels, cross-linked films and electrospun fibers containing hydrolyzed α-elastin all show preferable vascular material properties including regulation of SMC phenotype and increased mechanical elasticity. Electrospun materials are of particular interest as architecturally, these materials closely mimic the dimensions of elastic fibers *in vivo* (Li and Xia 2004).

Hydrolyzed elastin materials have also been proposed for use in the repair of elastic cartilage. In porous PCL scaffolds, infusion of α-elastin demonstrates enhanced scaffold elasticity and attachment and proliferation of articular cartilage chondrocytes *in vitro* (Annabi, Fathi et al. 2011). Replication of auricular-like cartilage has also been explored using alginate, collagen type I and κ-elastin containing hydrogels with auricular cartilage chondrocytes (de Chalain, Phillips et al. 1999). When these materials were implanted in mice and harvested after 12 weeks, matrix components including collagen and elastic fibers were present.

Dermal replacements containing hydrolyzed elastin demonstrate improved properties over elastin-free materials in regards to wound contraction and tissue regeneration (Rnjak, Wise et al. 2011). For example, MatriDerm, a collagen based scaffold with α-elastin shows improved skin elasticity (Ryssel, Gazyakan et al. 2008). Hydrogels formed exclusively from α-elastin (Figure 3) favorably support attachment and proliferation of dermal fibroblasts *in vitro* (Annabi, Mithieux et al. 2009; Annabi, Mithieux et al. 2009).

Scaffold	Advantages	Limitations	Reference
α-elastin film	-Low elastic modulus -attachment & proliferation of SMCs	-reduced SMC proliferation compared to TCPS	(Leach, Wolinsky et al. 2005)
elastin/gelatin gel	-Young's modulus matched to native artery -proliferation & infiltration of SMCs	-reduced SMC growth compared to TCPS	(Lamprou, Zhdan et al. 2010)
Collagen type I gels containing α-elastin	-SMC proliferation inhibited	- EC proliferation inhibited at high α-elastin concentrations	(Ito, Ishimaru et al. 1997)
α-elastin & collagen electrospun blended conduit	-attachment & proliferation of SMCs	-no mechanical testing	(Buttafoco, Kolkman et al. 2006)
α-elastin electrospun sheet	-SMC proliferation inhibited -α-SMA expression observed	-no mechanical testing	(Miyamoto, Atarashi et al. 2009)
α-elastin electrospun fibers	-attachment & proliferation of embryonic mesenchymal cells	-complete 3D constructs not created	(Li, Mondrinos et al. 2005)
α-elastin, PLGA & gelatin electrospun sheet	-mechanical properties tuned to artery through polymer content -proliferation of ECs on scaffold surface & infiltration of SMCs. -expression of functional EC molecules	-mechanical properties tested on electrospun sheets, not tubes	(Han, Lazarovici et al. 2011)
α-elastin, collagen type I & PLGA electrospun conduit	-matched compliance to bovine iliac artery -proliferation of ECs on inner & SMCs on outer surface of conduit -no immune reaction when implanted in mice	-scaffold contraction *in vitro*	(Stitzel, Liu et al. 2006; Lee, Yoo et al. 2007)
α-elastin, collagen type I & PLLA, PCL or PLCL blended electrospun conduit	-growth of bovine ECs -infiltration and α-SMA expression of SMCs	-scaffold contraction of PLCL blends *in vitro*	(Lee, Yoo et al. 2007)

Scaffold	Advantages	Limitations	Reference
α-elastin & PDO blended electrospun conduit	-mechanical properties matched to femoral artery with increased elastin content -increased cell infiltration with increased elastin content -increased graft burst pressure with suture reinforcement	-suture reinforcement lowers compliance	(Sell, McClure et al. 2006; Smith, McClure et al. 2008)
Elastin, collagen type I & collagen type III tri-layered electrospun conduit	-growth of EC, SMC and Fb in separate layers	-delamination of layers -no mechanical testing	(Boland, Matthews et al. 2004)
α-elastin, gelatin & PDS blended electrospun conduit	-matched tensile properties & elastic modulus to femoral artery	-loss of tensile properties due to *in vitro* degradation -no cell studies performed	(Thomas, Zhang et al. 2009)
α-elastin, gelatin & Maxon multi-layered electrospun conduit	-comparable mechanical properties to femoral artery	-no cell studies performed	(Thomas, Zhang et al. 2007)
bovine elastin, PGC, PCL & gelatin bi-layered electrospun conduit	-tensile strength matched to native artery -attachment & proliferation of EC & EPCs	-no SMC characterization	(Zhang, Thomas et al. 2010; Zhang, Thomas et al. 2010; Zhang, Xu et al. 2011)
α-elastin, collagen, PCL tri-layered electrospun conduit	-mechanical properties matched to native artery by modulation of elastin & PCL content	-no cell studies performed	(McClure, Sell et al. 2010)

Table 1. Scaffolds for vascular constructs synthesized using hydrolyzed elastin.
Abbreviations: SMC: smooth muscle cell, EC: endothelial cell, EPC: endothelial progenitor cells, Fb: fibroblast, TCPS: tissue culture polystyrene, PLGA: poly(D,L-lactide-*co*-glycolide), PLLA: poly(L-lactide), PCL: polycaprolactone, PLCL: poly(L-lactide-*co*-ε-caprolactone), PDO: polydioxanone, PDS: polydioxanone, PGC: poliglecaprone

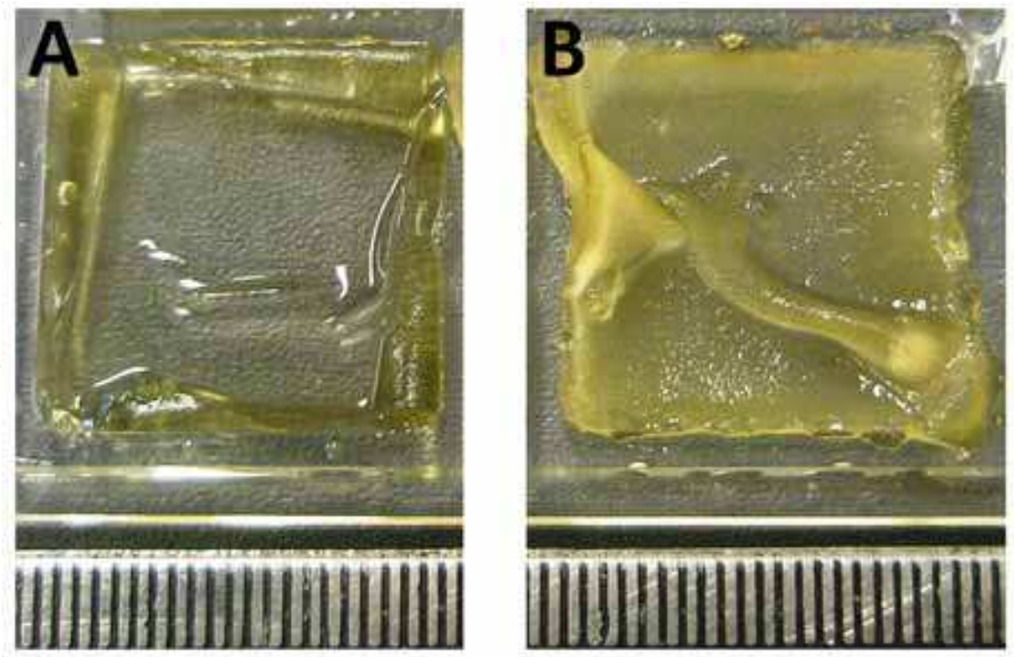

Fig. 3. Examples of α-elastin hydrogels that were synthesized by cross-linking with (A) 0.05% and (B) 0.1% (w/v) glutaraldehyde

8. Elastin-sequence based materials

8.1 Synthetic elastin-based peptides

Synthetic peptides based on key elastin sequences present elastin-like properties including self-assembly, cross-linking and cell interactions (Long, King et al. 1989; Faury, Garnier et al. 1998; Bellingham, Woodhouse et al. 2001; Karnik, Brooke et al. 2003; Karnik, Wythe et al. 2003). Coating of materials with elastin peptides can improve biocompatibility by providing protein sequences required for cell binding (reviewed by (Almine, Bax et al. 2010)). Some three dimensional materials formed from elastin-based peptides demonstrate elastin-like properties, including hydrogels that support cell growth and possess high degrees of elasticity (Keeley, Bellingham et al. 2002; Trabbic-Carlson, Setton et al. 2003). However as with hydrolyzed elastin preparations, synthetic peptides can lack the full repertoire of properties of the fully intact protein and are associated with inflammation (Faury, Ristori et al. 1995).

8.2 Recombinant human tropoelastin

Recombinant human tropoelastin (rhTE) is expressed and purified can be made as a recombinant protein in *Escherichia coli*. rhTE exhibits many properties of native tropoelastin including the ability to coacervate under physiological conditions and be cross-linked *in vitro* to form insoluble elastin fibers (Vrhovski, Jensen et al. 1997; Muiznieks, Jensen et al. 2003). rhTE promotes endothelial cell and fibroblast attachment, spreading and proliferation when used as a surface coating (Bax, Rodgers et al. 2009; Rnjak, Li et al. 2009; Wise, Byrom et al. 2011) and improves the biocompatibility of implanted devices (Yin, Wise et al. 2009; Wilson, Gibson et al. 2010).

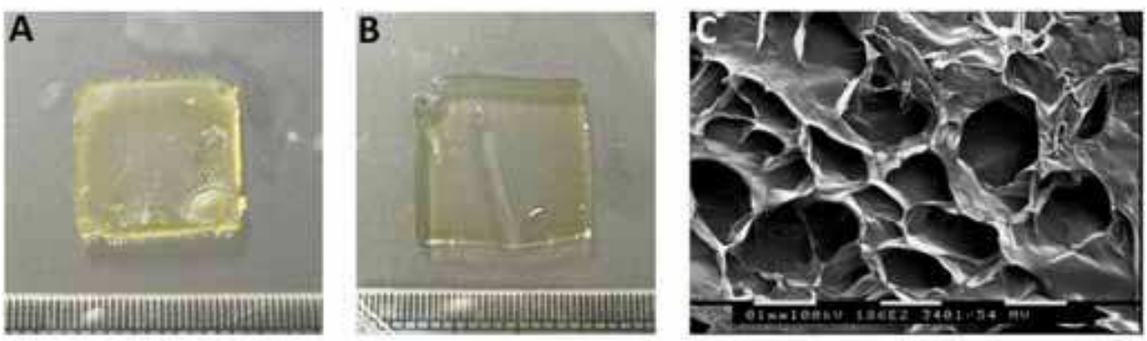

Fig. 4. Synthetic human elastin hydrogels (A) formed from the cross-linking of rhTE and (B) after hydration in phosphate buffered saline. (C) Hydrogel surface porosity shown by scanning electron microscopy

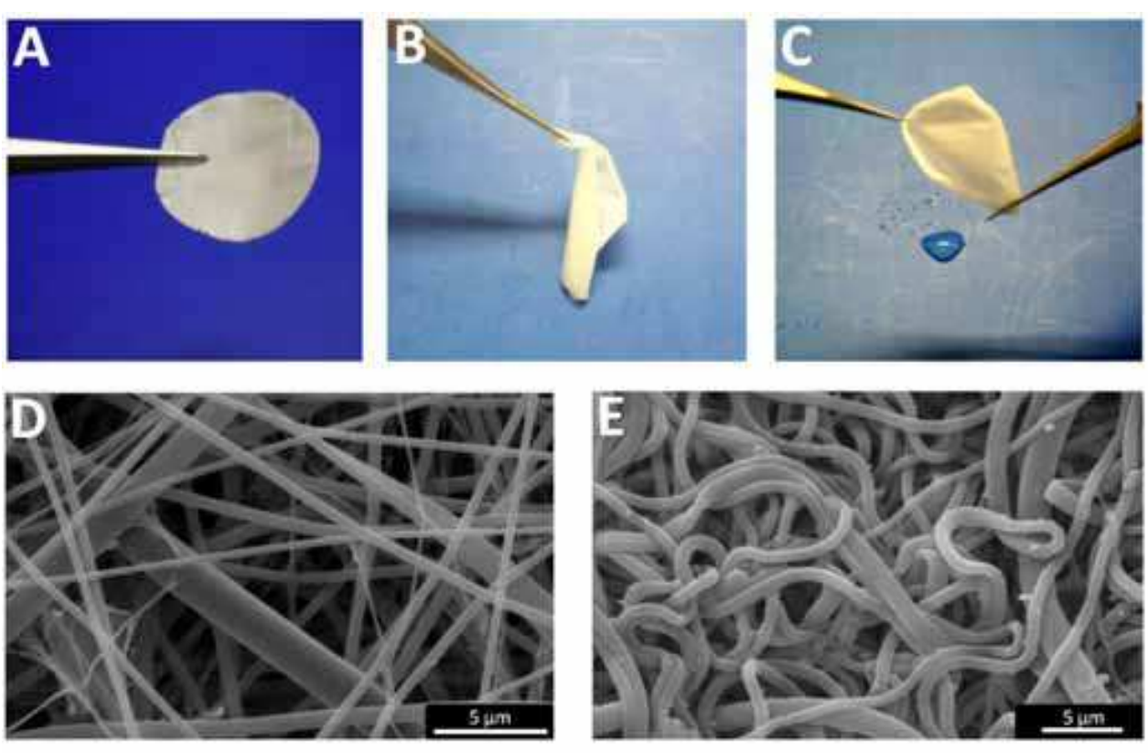

Fig. 5. Synthetic elastin electrospun materials.
Representative photographs of a synthetic human elastin scaffold (A) before cross-linking and (B-C) after cross-linking with hexamethylene diisocyanate and wetting with phosphate buffered saline. Uncross-linked scaffolds are stiff and inflexible while cross-linked scaffolds are highly flexible and collapse when not supported. Scanning electron micrographs of (D) uncross-linked electrospun fibers and (E) cross-linked electrospun fibers reveal the ribbon-like morphology of fibers

Three dimensional biomaterials are produced by cross-linking rhTE to form synthetic human elastin. Synthetic elastin has advantages over decellularized tissue and hydrolyzed elastin

preparations as it utilizes human protein avoiding potential problems arising from species differences while benefiting from homogeneity to improve reproducibility and uniformity.

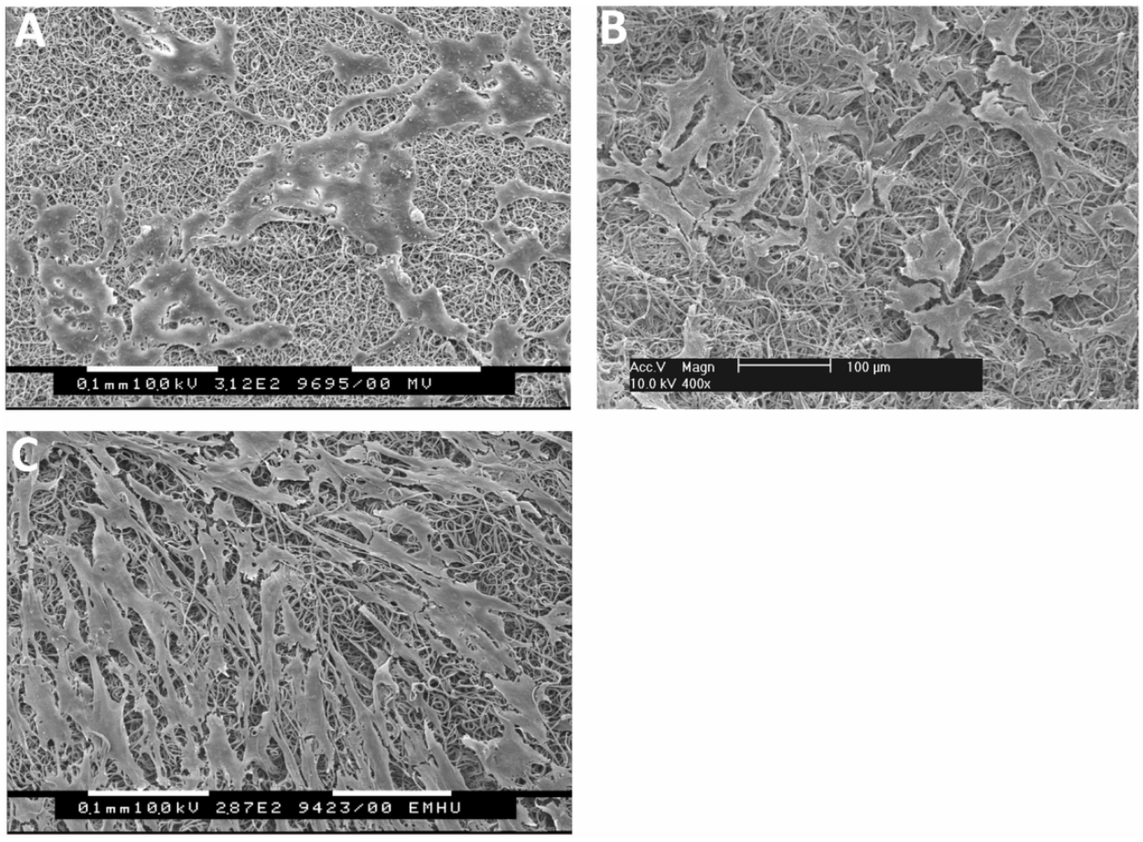

Fig. 6. Examples of synthetic elastin electrospun materials.
Scanning electron micrographs of (A) human umbilical vein endothelial cells, (B) SMCs and (C) dermal fibroblasts cultured on synthetic elastin fibers

These types of synthetic elastin hydrogels can be made by chemical cross-linking (Mithieux, Rasko et al. 2004), enzyme treatment (Mithieux, Wise et al. 2005) or raising the pH (Mithieux, Tu et al. 2009) of rhTE solutions (Figure 4). The hydrogels demonstrate mechanical properties that are consistent with native elastin including low elastic moduli, support of attachment and proliferation of dermal fibroblasts (Mithieux, Rasko et al. 2004; Rnjak, Li et al. 2009; Annabi, Mithieux et al. 2010). Increases in hydrogel porosity using high pressure CO_2 or the incorporation of glycosaminoglycans improve cell infiltration into hydrogels (Annabi, Mithieux et al. 2010; Tu, Mithieux et al. 2010) where the maintenance of fibroblasts within these scaffolds present them as candidate dermal substitutes.
Electrospun synthetic elastin allows for the formation of highly organized biomaterials with tunable mechanical biological properties. Electrospun synthetic elastin is formed by the electrospinning and chemical cross-linking of rhTE to yield ribbon-like microfibers (Figure 5) whose dimensions match those of native elastin fibers (Nivison-Smith, Rnjak et al. 2010). Highly porous electrospun synthetic elastin scaffolds, generated by using high flow rates facilitate the infiltration of dermal fibroblasts *in vitro* and present an alternative to synthetic elastin hydrogels as a dermal replacement (Rnjak, Li et al. 2009).

As a potential vascular material, electrospun synthetic elastin shows attractive characteristics including internal mammary artery-matched elastic mechanical properties, low platelet adhesion (Wise, Byrom et al. 2011) and support of growing human vascular cells including SMCs, endothelial cells (Figure 6) and embryonic palatal mesenchymal stem cells (Li, Mondrinos et al. 2005; Nivison-Smith, Rnjak et al. 2010). Synthetic human elastin fibers can also direct cell spreading to resemble cell organization *in vivo*. For example, the radial alignment of SMC in the arterial media is mimicked by culture of these cells on parallel synthetic elastin fibers (Nivison-Smith & Weiss 2011, *submitted*). Blended conduits of synthetic elastin and silk or polycaprolactone display elasticity and cell adhesion properties courtesy of the rhTE component while the composite component confers additional mechanical strength (Hu, Wang et al. 2010; Wise, Byrom et al. 2011).

9. Conclusion

Elastin is an essential matrix protein, so it is logical that biomaterials designed for elastic tissues should incorporated elastin. Difficulties in sourcing pure intact elastin preparations, particularly those that reflect human sequences, has limited the generation of these materials. Synthetic human elastin that is made from rhTE presents a versatile and stable component of vascular and dermal materials. Elastin-based constructs demonstrate mechanical and biological properties consistent with native elastin and have potential for a wider range of applications.

10. Acknowledgements

We acknowledge grant support from the Australian Research Council and the National Health and Medical Research Council. We thank Dr. Anna Waterhouse for the images related to elastin hydrogels. We acknowledge the contributions of the staff and the facilities made available at the AMMRF (Australian Microscopy & Microanalysis Research Facility) at the Australian Center for Microscopy and Microanalysis, The University of Sydney.

11. References

Abbott, W. M., J. Megerman, et al. (1987). Effect of compliance mismatch on vascular graft patency. *JVasc Surg* 5(2): 376-382.

Akhtar, K., T. J. Broekelmann, et al. (2011). Oxidative modifications of the C-terminal domain of tropoelastin prevent cell binding. *JBiol Chem*.

Almine, J. F., D. V. Bax, et al. (2010). Elastin-based materials. *Chem Soc Rev* 39(9): 3371-3379.

Annabi, N., A. Fathi, et al. (2011). The effect of elastin on chondrocyte adhesion and proliferation on poly (varepsilon-caprolactone)/elastin composites. *Biomaterials* 32(6): 1517-1525.

Annabi, N., S. M. Mithieux, et al. (2009). Synthesis of highly porous crosslinked elastin hydrogels and their interaction with fibroblasts in vitro. *Biomaterials* 30(27): 4550-4557.

Annabi, N., S. M. Mithieux, et al. (2009). The fabrication of elastin-based hydrogels using high pressure CO(2). *Biomaterials* 30(1): 1-7.

Annabi, N., S. M. Mithieux, et al. (2010). Cross-linked open-pore elastic hydrogels based on tropoelastin, elastin and high pressure CO2. *Biomaterials* 31(7): 1655-1665.

Baldock, C., A. F. Oberhauser, et al. (2011). Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity. *Proc Natl Acad Sci U S A* 108(11): 4322-4327.

Barbie, C., C. Angibaud, et al. (1989). Some factors affecting properties of elastin-fibrin biomaterial. *Biomaterials* 10(7): 445-448.

Bax, D. V., U. R. Rodgers, et al. (2009). Cell adhesion to tropoelastin is mediated via the C-terminal GRKRK motif and integrin alphaVbeta3. *JBiol Chem* 284(42): 28616-28623.

Bellingham, C. M., K. A. Woodhouse, et al. (2001). Self-aggregation characteristics of recombinantly expressed human elastin polypeptides. *Biochim Biophys Acta* 1550(1): 6-19.

Boland, E. D., J. A. Matthews, et al. (2004). Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front Biosci* 9: 1422-1432.

Broekelmann, T. J., B. A. Kozel, et al. (2005). Tropoelastin interacts with cell-surface glycosaminoglycans via its COOH-terminal domain. *J Biol Chem* 280(49): 40939-40947.

Brooke, B. S., A. Bayes-Genis, et al. (2003). New insights into elastin and vascular disease. *Trends Cardiovasc Med* 13(5): 176-181.

Buijtenhuijs, P., L. Buttafoco, et al. (2004). Tissue engineering of blood vessels: characterization of smooth-muscle cells for culturing on collagen-and-elastin-based scaffolds. *Biotechnol Appl Biochem* 39(Pt 2): 141-149.

Buttafoco, L., P. Engbers-Buijtenhuijs, et al. (2006). First steps towards tissue engineering of small-diameter blood vessels: preparation of flat scaffolds of collagen and elastin by means of freeze drying. *JBiomed Mater Res B Appl Biomater* 77(2): 357-368.

Buttafoco, L., N. G. Kolkman, et al. (2006). Electrospinning of collagen and elastin for tissue engineering applications. *Biomaterials* 27(5): 724-734.

Callewaert, B., M. Renard, et al. (2011). New insights into the pathogenesis of autosomal-dominant cutis laxa with report of five ELN mutations. *Hum Mutat* 32(4): 445-455.

Chlupac, J., E. Filova, et al. (2009). Blood vessel replacement: 50 years of development and tissue engineering paradigms in vascular surgery. *Physiol Res* 58 Suppl 2: S119-139.

Chuang, T. H., C. Stabler, et al. (2009). Polyphenol-stabilized tubular elastin scaffolds for tissue engineered vascular grafts. *Tissue Eng Part A* 15(10): 2837-2851.

Conklin, B. S., E. R. Richter, et al. (2002). Development and evaluation of a novel decellularized vascular xenograft. *Med Eng Phys* 24(3): 173-183.

Daamen, W. F., H. T. van Moerkerk, et al. (2003). Preparation and evaluation of molecularly-defined collagen-elastin-glycosaminoglycan scaffolds for tissue engineering. *Biomaterials* 24(22): 4001-4009.

Daamen, W. F., J. H. Veerkamp, et al. (2007). Elastin as a biomaterial for tissue engineering. *Biomaterials* 28(30): 4378-4398.

Dahl, S. L., J. Koh, et al. (2003). Decellularized native and engineered arterial scaffolds for transplantation. *Cell Transplant* 12(6): 659-666.

de Chalain, T., J. H. Phillips, et al. (1999). Bioengineering of elastic cartilage with aggregated porcine and human auricular chondrocytes and hydrogels containing alginate, collagen, and kappa-elastin. *JBiomed Mater Res* 44(3): 280-288.

De Vries, H. J., J. E. Zeegelaar, et al. (1995). Reduced wound contraction and scar formation in punch biopsy wounds. Native collagen dermal substitutes. A clinical study. *Br J Dermatol* 132(5): 690-697.

Engbers-Buijtenhuijs, P., L. Buttafoco, et al. (2005). Analysis of the balance between proliferation and apoptosis of cultured vascular smooth muscle cells for tissue-engineering applications. *Tissue Eng* 11(11-12): 1631-1639.

Faury, G., S. Garnier, et al. (1998). Action of tropoelastin and synthetic elastin sequences on vascular tone and on free Ca2+ level in human vascular endothelial cells. *Circ Res* 82(3): 328-336.

Faury, G., M. T. Ristori, et al. (1994). Role of the elastin-laminin receptor in the vasoregulation. *C R Acad Sci III* 317(9): 807-811.

Faury, G., M. T. Ristori, et al. (1995). Effect of elastin peptides on vascular tone. *J Vasc Res* 32(2): 112-119.

Gilbert, T. W., T. L. Sellaro, et al. (2006). Decellularization of tissues and organs. *Biomaterials* 27(19): 3675-3683.

Glagov, S., R. Vito, et al. (1992). Micro-architecture and composition of artery walls: relationship to location, diameter and the distribution of mechanical stress. *J Hypertens Suppl* 10(6): S101-104.

Hafemann, B., S. Ensslen, et al. (1999). Use of a collagen/elastin-membrane for the tissue engineering of dermis. *Burns* 25(5): 373-384.

Han, J., P. Lazarovici, et al. (2011). Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering. *Biomacromolecules* 12(2): 399-408.

Hinek, A. (1995). The 67 kDa spliced variant of beta-galactosidase serves as a reusable protective chaperone for tropoelastin. *Ciba Found Symp* 192: 185-191; discussion 191-186.

Holst, J., S. Watson, et al. (2010). Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. *Nat Biotechnol* 28(10): 1123-1128.

Hornebeck, W., J. M. Tixier, et al. (1986). Inducible adhesion of mesenchymal cells to elastic fibers: elastonectin. *Proc Natl Acad Sci U S A* 83(15): 5517-5520.

Hu, X., X. Wang, et al. (2010). Biomaterials derived from silk-tropoelastin protein systems. *Biomaterials* 31(32): 8121-8131.

Hucthagowder, V., E. Morava, et al. (2009). Loss-of-function mutations in ATP6V0A2 impair vesicular trafficking, tropoelastin secretion and cell survival. *Hum Mol Genet* 18(12): 2149-2165.

Indik, Z., W. R. Abrams, et al. (1990). Production of recombinant human tropoelastin: characterization and demonstration of immunologic and chemotactic activity. Arch *Biochem Biophys* 280(1): 80-86.

Indik, Z., H. Yeh, et al. (1987). Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proc Natl Acad Sci U S A* 84(16): 5680-5684.

Ito, S., S. Ishimaru, et al. (1997). Inhibitory effect of type 1 collagen gel containing alpha-elastin on proliferation and migration of vascular smooth muscle and endothelial cells. *Cardiovasc Surg* 5(2): 176-183.

Ito, S., S. Ishimaru, et al. (1998). Effect of coacervated alpha-elastin on proliferation of vascular smooth muscle and endothelial cells. *Angiology* 49(4): 289-297.

Jacob, M. P. and W. Hornebeck (1985). Isolation and characterisation of insoluble and kappa-elastins. *Methods of connective tissue research* L. Robert, M. Moczar and E. Moczar. Basel, Karger. 4: 92-129.

Jung, S., J. T. Rutka, et al. (1998). Tropoelastin and elastin degradation products promote proliferation of human astrocytoma cell lines. *J Neuropathol Exp Neurol* 57(5): 439-448.

Kagan, H. M. and K. A. Sullivan (1982). Lysyl oxidase: preparation and role in elastin biosynthesis. *Methods Enzymol* 82 Pt A: 637-650.

Kamisato, S., Y. Uemura, et al. (1997). Involvement of intracellular cyclic GMP and cyclic GMP-dependent protein kinase in alpha-elastin-induced macrophage chemotaxis. *J Biochem 1*21(5): 862-867.

Karnik, S. K., B. S. Brooke, et al. (2003). A critical role for elastin signaling in vascular morphogenesis and disease. *Development* 130(2): 411-423.

Karnik, S. K., J. D. Wythe, et al. (2003). Elastin induces myofibrillogenesis via a specific domain, VGVAPG. *Matrix Biol* 22(5): 409-425.

Keeley, F. W., C. M. Bellingham, et al. (2002). Elastin as a self-organizing biomaterial: use of recombinantly expressed human elastin polypeptides as a model for investigations of structure and self-assembly of elastin. *Philos Trans R Soc Lond B Biol Sci* 357(1418): 185-189.

Kielty, C. M. (2006). Elastic fibres in health and disease. *Expert Rev Mol Med* 8(19): 1-23.

Koens, M. J., K. A. Faraj, et al. (2010). Controlled fabrication of triple layered and molecularly defined collagen/elastin vascular grafts resembling the native blood vessel. *Acta Biomater* 6(12): 4666-4674.

Lammers, G., G. S. Tjabringa, et al. (2009). A molecularly defined array based on native fibrillar collagen for the assessment of skin tissue engineering biomaterials. *Biomaterials* 30(31): 6213-6220.

Lamprou, D., P. Zhdan, et al. (2010). Gelatine and Gelatine/Elastin Nanocomposites for Vascular Grafts: Processing and Characterization. *JBiomater Appl.*

Leach, J. B., J. B. Wolinsky, et al. (2005). Crosslinked alpha-elastin biomaterials: towards a processable elastin mimetic scaffold. *Acta Biomater* 1(2): 155-164.

Lee, S. J., J. J. Yoo, et al. (2007). In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. *JBiomed Mater Res A* 83(4): 999-1008.

Li, D. and Y. N. Xia (2004). Electrospinning of nanofibers: Reinventing the wheel? *Advanced Materials* 16(14): 1151-1170.

Li, D. Y., B. Brooke, et al. (1998). Elastin is an essential determinant of arterial morphogenesis. *Nature* 393(6682): 276-280.

Li, M., M. J. Mondrinos, et al. (2005). Electrospun protein fibers as matrices for tissue engineering. *Biomaterials* 26(30): 5999-6008.

Lloyd-Jones, D., R. J. Adams, et al. (2010). Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation* 121(7): e46-e215.

Long, M. M., V. J. King, et al. (1989). Elastin repeat peptides as chemoattractants for bovine aortic endothelial cells. *JCell Physiol* 140(3): 512-518.

Macchiarini, P., P. Jungebluth, et al. (2008). Clinical transplantation of a tissue-engineered airway. *Lancet* 372(9655): 2023-2030.

McClure, M. J., S. A. Sell, et al. (2010). A three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen: a preliminary study. *Acta Biomater* 6(7): 2422-2433.

Mecham, R. P. (1991). Elastin synthesis and fiber assembly. *Ann N Y Acad Sci* 624: 137-146.

Mithieux, S. M., J. E. Rasko, et al. (2004). Synthetic elastin hydrogels derived from massive elastic assemblies of self-organized human protein monomers. *Biomaterials* 25(20): 4921-4927.

Mithieux, S. M., Y. Tu, et al. (2009). In situ polymerization of tropoelastin in the absence of chemical cross-linking. *Biomaterials* 30(4): 431-435.

Mithieux, S. M. and A. S. Weiss (2005). Elastin. *Adv Protein Chem* 70: 437-461.

Mithieux, S. M., S. G. Wise, et al. (2005). A model two-component system for studying the architecture of elastin assembly in vitro. *JStruct Biol* 149(3): 282-289.

Miyamoto, K., M. Atarashi, et al. (2009). Creation of cross-linked electrospun isotypic-elastin fibers controlled cell-differentiation with new cross-linker. *Int JBiol Macromol* 45(1): 33-41.

Mochizuki, S., B. Brassart, et al. (2002). Signaling pathways transduced through the elastin receptor facilitate proliferation of arterial smooth muscle cells. *JBiol Chem* 277(47): 44854-44863.

Muiznieks, L. D., S. A. Jensen, et al. (2003). Structural changes and facilitated association of tropoelastin. *Arch Biochem Biophys* 410(2): 317-323.

Nivison-Smith, L., J. Rnjak, et al. (2010). Synthetic human elastin microfibers: stable cross-linked tropoelastin and cell interactive constructs for tissue engineering applications. *Acta Biomater* 6(2): 354-359.

Partridge, S. M., H. F. Davis, et al. (1955). The chemistry of connective tissues. 2. Soluble proteins derived from partial hydrolysis of elastin. *Biochem J*61(1): 11-21.

Pasquali-Ronchetti, I. and M. Baccarani-Contri (1997). Elastic fiber during development and aging. *Microsc Res Tech* 38(4): 428-435.

Petersen, T. H., E. A. Calle, et al. (2010). Tissue-engineered lungs for in vivo implantation. *Science* 329(5991): 538-541.

Price, A. P., K. A. England, et al. (2010). Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. *Tissue Eng Part A* 16(8): 2581-2591.

Rnjak, J., Z. Li, et al. (2009). Primary human dermal fibroblast interactions with open weave three-dimensional scaffolds prepared from synthetic human elastin. *Biomaterials* 30(32): 6469-6477.

Rnjak, J., S. G. Wise, et al. (2011). Severe Burn Injuries and the Role of Elastin in the Design of Dermal Substitutes. *Tissue Eng Part B Rev*.

Rodgers, U. R. and A. S. Weiss (2004). Integrin alpha v beta 3 binds a unique non-RGD site near the C-terminus of human tropoelastin. *Biochimie* 86(3): 173-178.

Rodgers, U. R. and A. S. Weiss (2005). Cellular interactions with elastin. *Pathol Biol (Paris)* 53(7): 390-398.

Rosenbloom, J., W. R. Abrams, et al. (1993). Extracellular matrix 4: the elastic fiber. *FASEB J* 7(13): 1208-1218.

Roten, S. V., S. Bhat, et al. (1996). Elastic fibers in scar tissue. *JCutan Pathol* 23(1): 37-42.

Ryssel, H., E. Gazyakan, et al. (2008). The use of MatriDerm in early excision and simultaneous autologous skin grafting in burns--a pilot study. *Burns* 34(1): 93-97.

Sandberg, L. B., N. T. Soskel, et al. (1981). Elastin structure, biosynthesis, and relation to disease states. *N Engl JMed* 304(10): 566-579.

Schmidt, C. E. and J. M. Baier (2000). Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials* 21(22): 2215-2231.

Sell, S. A., M. J. McClure, et al. (2006). Electrospun polydioxanone-elastin blends: potential for bioresorbable vascular grafts. *Biomed Mater* 1(2): 72-80.

Senior, R. M., G. L. Griffin, et al. (1980). Chemotactic activity of elastin-derived peptides. J *Clin Invest* 66(4): 859-862.

Shapiro, S. D., S. K. Endicott, et al. (1991). Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon. *JClin Invest* 87(5): 1828-1834.

Smith, M. J., M. J. McClure, et al. (2008). Suture-reinforced electrospun polydioxanone-elastin small-diameter tubes for use in vascular tissue engineering: a feasibility study. *Acta Biomater* 4(1): 58-66.

Starcher, B. (2001). A ninhydrin-based assay to quantitate the total protein content of tissue samples. *Anal Biochem* 292(1): 125-129.

Stitzel, J., J. Liu, et al. (2006). Controlled fabrication of a biological vascular substitute. *Biomaterials* 27(7): 1088-1094.

Thomas, V., X. Zhang, et al. (2007). Functionally graded electrospun scaffolds with tunable mechanical properties for vascular tissue regeneration. *Biomed Mater* 2(4): 224-232.

Thomas, V., X. Zhang, et al. (2009). A biomimetic tubular scaffold with spatially designed nanofibers of protein/PDS bio-blends. *Biotechnol Bioeng* 104(5): 1025-1033.

Trabbic-Carlson, K., L. A. Setton, et al. (2003). Swelling and mechanical behaviors of chemically cross-linked hydrogels of elastin-like polypeptides. *Biomacromolecules* 4(3): 572-580.

Tu, Y., S. M. Mithieux, et al. (2010). Synthetic elastin hydrogels that are coblended with heparin display substantial swelling, increased porosity, and improved cell penetration. *JBiomed Mater Res A* 95(4): 1215-1222.

Uchimura, E., Y. Sawa, et al. (2003). Novel method of preparing acellular cardiovascular grafts by decellularization with poly(ethylene glycol). *J Biomed Mater Res A* 67(3): 834-837.

Uitto, J., A.M. Christiano, V.M. Kahari, M.M. Bashir, and J. Rosenbloom, *Molecular biology and pathology of human elastin.* Biochem Soc Trans, 1991. 19(4): p. 824-9

Umeda, H., F. Nakamura, et al. (2001). Oxodesmosine and isooxodesmosine, candidates of oxidative metabolic intermediates of pyridinium cross-links in elastin. *Arch Biochem Biophys* 385(1): 209-219.

Urban, Z., J. Zhang, et al. (2001). Supravalvular aortic stenosis: genetic and molecular dissection of a complex mutation in the elastin gene. *Hum Genet* 109(5): 512-520.

Vrhovski, B., S. Jensen, et al. (1997). Coacervation characteristics of recombinant human tropoelastin. *Eur JBiochem* 250(1): 92-98.

Vrhovski, B. and A. S. Weiss (1998). Biochemistry of tropoelastin. *Eur JBiochem* 258(1): 1-18.

Williamson, M. R., A. Shuttleworth, et al. (2007). The role of endothelial cell attachment to elastic fibre molecules in the enhancement of monolayer formation and retention, and the inhibition of smooth muscle cell recruitment. *Biomaterials* 28(35): 5307-5318.

Wilson, B. D., C. C. Gibson, et al. (2010). Novel Approach for Endothelializing Vascular Devices: Understanding and Exploiting Elastin-Endothelial Interactions. *Ann Biomed Eng*.

Wise, S. G., M. J. Byrom, et al. (2011). A multilayered synthetic human elastin/polycaprolactone hybrid vascular graft with tailored mechanical properties. *Acta Biomater* 7(1): 295-303.

Wissink, M. J., M. J. van Luyn, et al. (2000). Endothelial cell seeding on crosslinked collagen: effects of crosslinking on endothelial cell proliferation and functional parameters. *Thromb Haemost* 84(2): 325-331.

Yin, Y., S. G. Wise, et al. (2009). Covalent immobilisation of tropoelastin on a plasma deposited interface for enhancement of endothelialisation on metal surfaces. *Biomaterials* 30(9): 1675-1681.

Zhang, X., V. Thomas, et al. (2010). Two ply tubular scaffolds comprised of proteins/poliglecaprone/polycaprolactone fibers. *J Mater Sci Mater Med* 21(2): 541-549.

Zhang, X., V. Thomas, et al. (2010). An in vitro regenerated functional human endothelium on a nanofibrous electrospun scaffold. *Biomaterials* 31(15): 4376-4381.

Zhang, X., Y. Xu, et al. (2011). Engineering an antiplatelet adhesion layer on an electrospun scaffold using porcine endothelial progenitor cells. *J Biomed Mater Res A*.

Zou, Y. and Y. Zhang (2009). An experimental and theoretical study on the anisotropy of elastin network. *Ann Biomed Eng* 37(8): 1572-1583.

Regenerative Medicine and Tissue Engineering - Cells and Biomaterials

Edited by Prof. Daniel Eberli

ISBN 978-953-307-663-8
Hard cover, 588 pages
Publisher InTech
Published online 29, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

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