Analysis of the Acellular Matrix, Growth Factors, and Cytokines Present in ArthroFLEX® Allograft

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Objective

Identify the extracellular matrix (ECM) components, growth factors, and cytokines present in ArthroFlex decellularized, sterile human dermal allograft.

Introduction

Surgically reattached tendons often heal improperly by forming weaker fibrous connections between the muscle and bone. They do not recover full mechanical strength and display a high rate of retear.[1-4] Acellular human dermis can aid in the repair of torn tendons by providing supplemental strength and structural integrity to the reattachment site. Human dermis is a complex tissue containing various extracellular matrix molecules, growth factors, and cytokines.[5] The purpose of this study was to ensure that ArthroFlex allograft, a minimally manipulated human dermis product, retains the biological components that provide supplemental strength and that can structurally support the repair of reattached tendons.

Methodology

Protein Analysis

Samples were solubilized in a detergent solution assisted by mechanical homogenization; this was followed by protein separation based on molecular size. Subsequently, the separated proteins were enzymatically fragmented, after which the amino acid sequence of each fragment was determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS). In LC-MS/MS, the fragmented proteins present in a solution are separated by molecular weight. Then, each separated fragment is further broken into smaller components and the molecular weights of those smaller components are determined. From the molecular weights of the smaller components, the amino acid sequence of the original fragment can be resolved. The amino acid sequences of each fragment were then compared against a database containing the sequences of known proteins to determine the corresponding protein for each fragment. From this comparison, a list of the proteins that corresponded to each fragment was generated. This list was mined for ECM components, growth factors, and cytokines to create a table of proteins whose fragments were found in the ArthroFlex allograft sample. Additionally, some components were further verified or identified by immunohistochemical staining and by enzyme-linked immunosorbent assay.[6]

Conclusion

The results of this study indicate that ArthroFlex allograft retains ECM components, matrikines, growth factors, and cytokines consistent with minimally manipulated human tissue and relevant to the structural support of damaged soft tissue. ArthroFlex allograft provides mechanical strength to surgically reattached tendons and the collagens that can supplement structural integrity. This can aid in the prevention of a retear.

Findings

Table 1: LC-MS/MS analysis found fragments of the following proteins in ArthroFlex dermal allograft.

Collagens	GF-binding ECM	Additional ECM	Matrikines	Growth Factors	Cytokines
Type I	Heparan sulfate proteoglycan (HSPG)	Elastin	Tenascin-C	BMP6	IL1a
Type III	Chondroitin sulfate proteoglycan (CSPG)	Nidogen (entactin)	Laminins	CTGF	IL1b
Type IV	Perlecan (HSPG2)	Keratin	Decorin	EGF	IL2
Type V	Aggrecan		Endostatin	HGF	IL5
Type VI	Lumican		Pentastatin	PDGFD	IL9
Type VII	Versican		Tumstatin	TGFB1	IL22b
Type VIII	Glypican		Elastokines	VEGFA	IL25
Type XII	Syndecan			VEGFD (FIGF)	IL27
Type XIV	Tenascin (C and N)				IL32
Type XVII	Thrombospondin-2				TNF
Type XVIII	Dermatopontin				
Type XX	Decorin				
Type XXI	Vitronectin				
Type XXIII	Laminin (α1-5, β1-3, γ1-3)				
Type XXVII	Fibrinogen (fibrin precursor)				
Type XXVIII					

Discussion

Torn tendons surgically reattached to bone do not tend to regain their original mechanical strength and typically form an inferior scar tissue at the tendon-bone interface.[1,2] Tendon retear rates for rotator cuff repair surgeries range from 50 to 90% depending on the severity of the original tear, and are correlated with the functional outcome following repair.[3,4] ArthroFlex, a minimally manipulated acellular human dermal allograft, aids in the repair of torn tendons by providing additional strength to the tendon-bone integration site, potentially preventing retear. In an independent study, ArthroFlex allograft was demonstrated to increase the ultimate strength to failure of a reattached tendon compared to reattachment without the use of ArthroFlex allograft.[7]

The natural tendon repair process is characterized by the deposition of fibrous tissue at the tendon-bone interface, resulting in a tendon-bone attachment site that is weaker than the native insertion site.[4] When grafted on top of a tendon reattachment, ArthroFlex dermal allograft provides structural support to the repair site and a multitude of human-derived structural ECM proteins, including elastin and many types of collagen. Collagen and elastin provide strength and flexibility that are not properly recapitulated in the natural tendon healing process.

Chronic tendon injury and tendon retear is believed to be a byproduct of the proteases released following the apoptotic and autophagic cell death that occurs during injury.[2] This protease release results in a loop: a tendon injury causes cell death, the dead cells release proteases, the proteases weaken the tendon, and the tendon is left more susceptible to reinjury. LC-MS/MS analysis found that ArthroFlex dermal allograft contained ECM components present in the native dermis ECM, including collagens, proteoglycans, and elastin. ECM can modify the wound environment by providing the substrates for the proteases that are known to weaken the healing tendon and believed to be responsible for chronic tendon injury.

ArthroFLEX Allograft Provides Human ECM Support With a 10^{-6} Sterility Assurance Level

These findings suggest that ArthroFlex allograft retains a broad array of extracellular matrix components, matrikines, growth factors, and cytokines present in healthy human skin and provides structural ECM components that can help prevent retearing of surgically reattached tendons. ArthroFlex dermal allograft is the only bio-implant for augmentation of tendon reattachment composed of natural human ECM with greater than 97% of the DNA removed and a minimal risk of infection, as well as a 10^{-6} sterility assurance level.

References

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