Biological aspects of rotator cuff healing

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Summary

Tendon tears of the rotator cuff show a high prevalence in today's population. Patients suffer from permanent pain and disability, and surgical reconstruction may be the only possibility for abatement. The complex process of tendon-bone healing leads to mechanically inferior scar-tissue, which often results in retears or non-healing. In the current literature, factors such as patients age, sex and fatty muscle infiltration are highly correlated to the presence of rotator cuff tears and the incidence of retears. To improve the tendon tissue quality after surgical reconstructions biologically based strategies with use of growth factors arouse more and more interest in the last years. However, to optimize the treatment of rotator cuff tears the biological background of tears and retears must be investigated in more detail. This article will elucidate different aspects that have an impact on rotator cuff healing and give a brief insight in tendon/ligament cell culture and animal studies focusing on growth factor treatments.

Key words: age, fatty infiltration, growth factors, rotator cuff rupture, sex, tenocytes

Introduction

The shoulder joint is composed of three bones and the rotator cuff as a group of four muscles and their tendons, allowing movement and providing stability. In general, tears of the rotator cuff cause symptoms, such as persistent pain, disability, decreased range of motion and strength and very often a surgical reconstruction is needed. Rotator cuff tears are dependent on different clinical, demographic or radiological determined risk factors. A possible biological or cel-

lular background of this correlation is not jet clarified. The main complication of rotator cuff tears is the high retear rate. Therefore the biological augmentation of the tendon-bone healing with growth factors was highly debated in the last years. Different growth factors were analyzed for a positive influence on tendon/ligament cell cultures or in animal studies. However, no treatment option with growth factor could be transferred to clinical practice so far.

Clinical problem: Rotator cuff tears and retears

Rotator cuff tears can be caused by injury resulting in an acute tear or by degeneration as a result of for example increasing age, repeated overload or impingement. Partial tears are often asymptomatic, but can develop to complete tears. Tears of the rotator cuff are a relevant clinical problem and reason for millions of physician visits annually in the US (1). A surgical reconstruction is very often indicated, but does not lead to complete regeneration of a normal tendon-bone insertion site, but rather to formation of a weaker scar tissue (2). Within the last years the surgical techniques were improved (3-6). The main complication for rotator cuff repair, however, represents non-healing or retear of the tendon insertion site depending on factors such as patients age, sex and grade of fatty muscle infiltration (1, 7-13). Despite these observed risk factors, surgical rotator cuff repair is carried out identically between different patient clusters with no respect to potential biological differences. A further limitation is the fact that the currently discussed risk factors are based upon clinical and radiographic studies of observative nature and cannot provide causal answers on the biologic differences between different patient subgroups.

Risk factors influencing tendon integrity and healing

The incidence and the outcome after rotator cuff reconstructions depend on different biological and clinical factors. As an important biological factor, the age of the patient is highly correlated to the incidence of tendon tears and retears (1, 8, 11, 13). A study analyzing individuals with an asymptomatic shoulder found that partial or full-thickness tears strongly increase after the age of 50, and 80% of individuals older than 80 years had an unrecognized rotator cuff tear (11). The incidence of retears increased after the age of 60 (8) and Boileau reported on only 43% of patients in which tendons completely healed after the age of 65 (7). Some authors observed an influence of sex (14, 15), while others did not (1, 11, 16). For the biological factor sex sexual hormones, such as estrogen, were hypothesized to influence the healing. It was found that estrogen affected the collagen synthesis after acute exercise in tendon connective tissue (17). Gender as a "social" factor, including differences such as personality traits, attitudes, behaviors,

and cultural background, might also influence the healing outcome. A 2011 published study found higher levels of pre- and post-operative disability in women with rotator cuff pathology. They concluded that the biological aspect sex and the "social" aspect gender, defined in this study as expectations, participation limitation, and satisfaction, are important for the seen differences (18). The effect of another "social" factor in terms of working activity on shoulder disorders was investigated by van Rijn et al. (19). The literature review revealed no association between the job and rotator cuff tears or suprascapular nerve compression, but a correlation between repetitive work, awkward postures and forceful exertion in work with subacromial impingement syndrome. It is discussed controversially in the literature if the subacromial impingement syndrome is a cause of rotator cuff tears or if it is the effect of rotator cuff diseases. The different theories of the impingement syndrome were discussed in detail by Campbell and Dunn (20). Nicotine consumption (21), obesity and diabetes (14, 22) negatively influence the healing after rotator cuff surgery. A correlation of Statin-medication, which is a cholesterol lowering drug, and tendon tears was also hypothesized (23). It was discussed that a chronic, low-grade inflammation, as seen in patients with obesity and diabetes, represents a prolonged disruptive factor of tendon healing. Reason for this may be a reduced number of circulating macrophages in the acute inflammation phase, which may result in a less effective early healing (22). Furthermore several radiological determined factors influence the healing outcome. The radiographic grade of fatty muscle infiltration (according to Goutallier) (24) is strongly correlated to the rate of retears and the clinical outcome after rotator cuff reconstructions (8-10, 12). Thereby a Goutallier grade of 1 was shown to be the cutoff between tendon integrity and recurrent tears (10). In addition, the initial tear size (according to Boileau) (7) and the tendon retraction (according to Patte) (25) are correlated with the clinical outcome (26-28). To get more information about the influence of several factors on the healing outcome, Chung et al. included 272 patients in a study and found a significantly increased failure rate depending on the lower bone mineral density (BMD), older age, female sex, larger tear size, higher fatty muscle infiltration, diabetes, and shorter acromiohumeral distance (14). After multivariate analysis, however, only the BMD and the fatty muscle infiltration were an independent determining factor. Also the rehabilitation program after rotator cuff surgery influences the healing outcome. For example, a supervised physical therapy seems to be superior compared to home exercise (29) and nurse invented T-bar device led to improved range of motion in patients with shoulder surgeries (30). Furthermore, the correlation of genetic factors and the incidence of tendon diseases were hypothesized (31), but specific "tendon pathology" genes could not be identified so far, since it is a multifactorial disease. The main challenge is to understand the gene interactions and signaling pathways involved in tendon diseases (32). These partially heterogeneous findings from the clinical evaluation of rotator cuff healing in relation to different clinical, biological and "social" aspects, as summarized in Figure 1, highlight the importance of more detailed investigations. In-depth epidemiological data on the influence of these conditions are missing, likely due to the fact that the patients cannot easily be grouped in only one category. It is unclear, which of these biological or clinical factors play the key role in rotator cuff regeneration. To answer this question, further studies also on the molecular and cellular background of rotator cuff tears and retears are urgently needed.

Biology of tendon healing

The healing of tendon tears is briefly summarized in the following: after injury of the tendon the repair process starts to restore normal function by reestablishing the tendon fibers. In the first phase, the inflammation occurs immediately after injury by the disruption of blood vessels and the formation of a hematoma. Cytokines are released and different cell types such as erythrocytes, platelets, neutrophils, monocytes and macrophages migrate to site of injury. The invading cells remove cell debris and secret further cytokines and extracellular matrix proteins. The released angiogenic factors in-
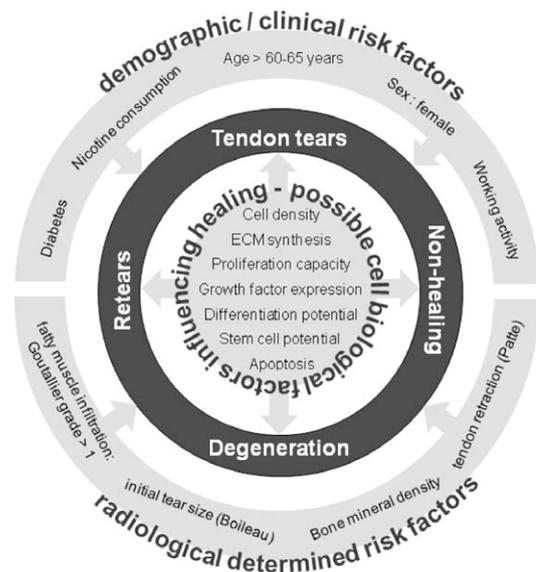

Figure 1. Demographic, clinical, radiological determined and possible cell biological risk factors influencing tendon healing outcome.

duce the formation of new vessels. Following this phase the proliferation of cells occur with further extracellular matrix production. During this proliferative phase an unorganized, highly cellularized and vascularized tissue forms that builds the initial scar tissue. At a later time point the remodeling phase starts. If the mechanical boundary conditions are given and the biology is not disturbed, the number of cells and vessels within this scar tissue decreases. The ratio between Collagen type I (Col-I) and Collagen type III (Col-III) fibers changes toward more Col-I and the fibers arrange longitudinally along the tendon axis, providing the mechanical strength of the tendon (33, 34). As a matter of course, numerous growth factors play an important role in the complex process of tendon healing, which is reviewed in detail by Oliva et al. (34). However, as mentioned earlier, the tendon

tissue shows only a repair and no regeneration, meaning a fully reconstruction of the original tissue does not occur. Little is known about the biology of retears, degeneration and non-healing of the rotator cuff. From a molecular perspective it was reported that torn or degenerated tendons show a higher amount of Col-III and a decreased amount of Col-I (35). Furthermore, degenerated tendons express higher amounts of cartilage markers such as Collagen type II (Col-II), aggrecan and sox9 and lower amounts of tendon related genes (36). Another study revealed that autophagic cell death, apoptosis and myofibroblast cell differentiation rise and cell density fall with tendon extracellular matrix (ECM) degeneration (37). In rat Achilles tendon, more rounded tenocyte nuclei and reduced number of tenocytes were found with increasing age (38). With increasing tear-size reparative and inflammatory processes, as well as the cellular activity of the tenocytes decreased, while chondroid redifferentiation and amyloid deposition increased (28). Furthermore a modified tissue structure (27) and more than 20% higher rates of apoptosis (39) were reported.

Growth factors for tendon/ligament healing

To improve the tendon tissue quality and biological response following surgical reconstructions, biologically based strategies have gained increasing interest over the past years. The most important cellular components in the tendon are tenocytes, but also tendon-derived stem cells or tendon progenitor cells were identified previously (40, 41). In general, the regenerative capacity of the tenocytes or tendon-derived stem cells (e.g. cell activity, proliferation, ECM synthesis) should be targeted. Tenocytes exhibit very low proliferative capacity and may benefit from biological support by growth factors. Therefore, it was suggested that the use of growth factors could improve tendon healing (42). But which growth factor should be chosen?

Positive *in vitro* and *in vivo* effects of growth factors such as Bone Morphogenetic Protein (BMP)-12 (43-46) or BMP-13 (47, 48), Transforming Growth Factor β (TGF-β) (49-52), Insulin-like Growth Factor I (IGF-I) (53), Platelet-derived Growth Factor (PDGF) (53-58), Growth and Differentiation Factor 5 (GDF-5) (59-61), Vascular Endothelial Growth Factor (VEGF) (54, 62, 63), and basic Fibroblast Growth Factor (bFGF) (49, 53-55, 64, 65) were shown. One of the main parameter analyzed in the *in vitro* experiments was the stimulation of cell proliferation and the expression or production of collagens of tendon fibroblasts. Additionally, *in vivo* an improved healing with increased biomechanical strength was found. So far no single growth factor treatment

for rotator cuff repair could be transferred to clinical use. However, autologous platelet-rich plasma (PRP) was, as a pilot study, clinically applied to patients who underwent arthroscopic rotator cuff repair surgeries by Randelli et al. (66, 67). The first study showed a reduced pain score and increased functional score compared to a pre-operative time point (66). The second study revealed a pain reduction for the PRP treated patients in the early postoperative time (until 30 days) compared to the non-PRP treated patient group, but no differences in functional scores and healing rates 6, 12, and 24 months after surgery were found (67). Further studies on the clinical PRP application in different fields of orthopedic surgery are reviewed by Foster et al. The critically evaluation shows also limitations of the studies, which often lack control groups or are limited in case series (68). Therefore, further clinical studies are necessary. A summary of studies regarding growth factor treatment of tendon cell cultures or tendon/ligament healing is shown in Table 1 and 2. However, this summary makes no claim to be complete. The mentioned studies focus not exclusively on cells isolated from tendons of the rotator cuff or rotator cuff repair, but include also studies on Anterior Cruciate Ligament (ACL), Patellar, Achilles or Flexor Tendon reconstructions.

The main complications for rotator cuff repair are retears at the tendon-bone insertion site. For tendon-bone healing of the rotator cuff it could therefore be useful to treat osteoblasts and tenocytes simultaneously. Therefore, the members of the TGF-β superfamily, BMP-2 and BMP-7, which are already clinically used for the treatment of bone defects, could be promising. Additionally to their positive effect on bone healing they seem to improve tendon healing, as it was reported in several *in vivo* studies: BMP-2 led to improved tendon-bone healing with increased biomechanical strength after anterior cruciate ligament (ACL) reconstructions (69, 70), flexor tendon reconstructions (71), or transplantation of the long digital extensor tendon to a bone tunnel (72). Similar positive effects were described for BMP-7 used in experimental ACL repair (73). In addition, several *in vitro* studies revealed effects of BMP-2 and BMP-7 on tendon cell cultures. Contrary results were observed for BMP-2 treatment on tenocytes. In a previous study, we found positive effects on Col-I expression and synthesis in tenocyte-like cells and a decrease in cell activity with use of BMP-2 in higher concentrations (1000ng/ml) (74). Other groups found no effects of BMP-2 (49, 54). BMP-7 application to ligament or tendon cell cultures resulted in a stimulated cell proliferation/activity and Col-I synthesis (74-77).

Studies on the effect of BMP-2 or BMP-7 on tenocytes of patient clusters differing in terms of age, sex or fatty muscle infiltration are not available.

Table 1. *In vitro* growth factor studies.

GF	Cell origin	Dosage	Effect	Ref.
BMP-2	Equine Superficial Digital FT	AdV transfection	Increased mineralization, AP expression, and Col-I synthesis	(45)
	Human SSP Tendon	100, 500, 1000ng/mL	Increased Col-I synthesis; decreased cell activity	(74)
	Murine Achilles Tendon cell lines	500ng/mL	Increased AP activity, AP and Osterix expression	(49)
	Canine FT	20, 50, 100ng/mL	No change of cell proliferation and Collagen production	(54)
BMP-7	Human SSP Tendon	100, 500,1000, 2000ng/mL	Increased cell activity, Col-I expression and synthesis	(74)
	Rat MCL	50, 100, 200, 300, 400ng/mL	Increased cell proliferation, AP activity, expression of Runx2/Cbfa1, Aggrecan, BMPR-IA, BMPR-II; decreased BMP-1, -2, -6 expression	(75)
	Bovine Digital Extensor Tendon	50, 100, 200ng/mL	Increased cell proliferation, DNA content, proteoglycan and Collagen synthesis	(76)
	Rat Achilles and Patellar Tendon	20, 50, 100, 200, 300, 400, 500ng/mL	Increased cell proliferation, Col-I, BMP-1, -3, GDF-1, -6, -8, -9, ActR-I, BMPR-IA expression; decreased SCX, Tendin, BMP-4, -6, -7 expression	(77)
BMP-12	Human Patellar Tendon	1, 10ng/mL	Increased cell proliferation, pro-Collagen-I, –III expression; decreased Decorin expression	(43)
	Chicken Tendon	AdV transfection	Increased Col-I synthesis	(44)
	Equine Superficial Digital FT	AdV transfection	Increased Col-I synthesis, COMP expression	(45)
BMP-13	human Patellar Tendon	1, 5, 10, 25, 50ng/mL	Increased cell proliferation, pro-Collagen type I; no change of Biglycan, Decorin, pro-Collagen type III expression	(47)
bFGF	Murine Achilles Tendon cell lines	5ng/mL	Increased cell proliferation	(49)
	Canine FT	10ng/mL	Increased cell proliferation, and Collagen production	(54)
	Rabbit Digitorum Profundus FT	0.5, 1, 5ng/mL	Increased cell proliferation	(53)
	Human RC Tendon	0.1, 1, 10, 100ng/mL	Increased cell proliferation, suppressed secretion of Collagens	(64)
	Canine FT	HBDS	Increased cell proliferation; Lubricin, HAS2, Decorin, MMP-1, -13 expression, decreased Col-I, -III expression	(55)
	Rat Intrasynovial tenocytes	AdV transfection	Increased Col-I, –III expression	(65)
IGF-I	Rabbit FT	10, 50, 100ng/mL	Increased cell proliferation	(53)
PDGF	Canine FT	10ng/mL	Increased cell proliferation, Collagen production	(54)
	Rabbit Digitorum Profundus FT	1, 10, 50ng/mL	Increased cell proliferation	(53)
	Canine FT	HBDS	Increased cell proliferation; decreased Col-I, -III expression	(55)
	Rat FT	Exogenous gene transfer	Increased Col-I expression	(58)
TGF-β	Murine Achilles Tendon cell lines	4ng/mL	Increased cell proliferation	(49)
	Rabbit FT	1, 5ng/mL TGF-β1, -2, -3	Increased Col-I, –III production	(50)
	Rabbit Tendon Sheath	Antisense gene transfer	Decreased Col-I, –III, TGF-β1 expression	(51)
VEGF	Canine FT	20, 50, 100ng/mL	No change of cell proliferation and Collagen production	(54)

Table 2. *In vivo* growth factor studies.

GF	Animal model	Doses	Effect	Ref.
BMP-2	Rabbit Digitorum Communis FT	15µg	Higher ultimate failure loads	(71)
	Rabbit ACL	AdV transfection	Increased stiffness and ultimate load to failure	(70)
	Canine LDET transplantation to bone tunnel	90, 180, 350, 680µg	Increased extensive bone formation, higher tendon pull-out strength	(72)
	Rabbit ISP Tendon	progenitor cell BMP-2 hydrogel	Increased fibrocartilage and bone layer, higher pull-out load	(78)
	Rabbit ACL	11.5, 50, 115µg	Increased new bone formation and stiffness, smaller tunnel diameter	(69)
BMP-7	Canine SSP model with titanium implant	3.5mg	Increased calcification, no differences in weight-bearing; stiffness, ultimate strength	(79)
	Ovine ACL	25µg	Increased new bone formation; greater strain resistance to force	(73)
BMP-12	Chicken Profundus FT	AdV transfection	Increased tensile strength and stiffness	(44)
	Ovine ISP Tendon	0.25mL of 0.35 mg/mL per hyaluronan paste	Increased maximum load and stiffness, accelerated healing	(46)
BMP-13	Rat SSP Tendon	AdV transfection	No change in new cartilage formation and Collagen fiber organization, stiffness, peak stress at failure or biomechanical strength	(48)
BMP-14	Rat Achilles Tendon	AdV transfection	Greater number of neotenocytes, greater tensile strength; no ectopic bone or cartilage formation	(80)
GDF-5	Rat Achilles Tendon	24, 55, 556ng/ cm suture	Higher ultimate tensile load and stiffness, Improved healing (histologic grading)	(59)
	Rabbit FT	55ng/cm suture	Better Soslowski histological Collagen score, better maximum load; no change in stiffness	(60)
	Rat Achilles Tendon	AdV transfection	Thicker tendons, greater cartilage formation; trend to higher strength	(61)
GF mix	Ovine ISP Tendon	1mg osteoind. bone extract	Increased new bone and fibrocartilage formation, improved load to failure	(81)
PDGF	Canine FT	100ng	Increased cell density, cell proliferation, Col-I expression, total DNA, reducible Collagen crosslinks	(56)
	Ovine ISP Tendon	75, 150, 500µg on collagen matrix	Increased load to failure and tendon to bone integration	(57)
TGF-β3	Rat SSP Tendon	HBDS, 50ng/matrix	Increased inflammation, cellularity, vascularity, cell proliferation, ultimate load, stiffness, toughness, ultimate stress, modulus	(52)
VEGF	Rat Achilles Tendon	5µg	Increased tensile strength, TGF-β expression	(62)
	Ovine ACL	tendon soaked in 5µg/mL solution	More newly formed vessels and infiltrative fibroblasts; reduced stiffness	(63)

AdV: Adenoviral
AP: Alkaline Phosphatase
COMP: Cartilage oligomeric matrix protein
FT: Flexor Tendon
GF: Growth factor
HBDS: Heparin-based delivery system

ISP: Infraspinatus
LDET: Long Digital Extensor Tendon
MCL: Medial Collateral Ligament
RC: Rotator Cuff
SCX: Scleraxis
SSP: Supraspinatus

Conclusion

Taken all together, the pathophysiology of tissue degeneration and retears of rotator cuff tendons is not yet clarified. It is obvious that tenocytes play an important role in rotator cuff tendon healing. For an optimal biologically based strategy it is of great importance to analyze the potential of the cells from different patient groups and their behavior following biological stimulation. A precisely addressed growth factor treatment together with an improved surgical technique could lead to improved osteofibroblastic integration of the tendon to the bony footprint. Potentially, certain patient clusters (for instance sex-dependent with higher age) might be associated with decreased biological activity, leading to insufficient regeneration, and might benefit from augmentation with growth factors.

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