

Enhanced reparative activities in human ACL lesions via pNaSS-grafted PCL film-guided rAAV-mediated overexpression of FGF-2 and TGF- β in an experimental humoral neutralization environment

Mostafa A Rabie^{1,2}, Jagadeesh K. Venkatesan¹, Gertrud Schmitt¹, Henning Madry¹, Véronique Migonney³, Magali Cucchiari¹

¹Center of Experimental Orthopaedics, Saarland University Medical Center, D-66421 Homburg/Saar, Germany

²Department of Pharmacology & Toxicology, Faculty of Pharmacy, Cairo University, 11562, Egypt

³LBPS/CSPBAT UMR CNRS 7244, Université Sorbonne Paris Nord, 93430 Villetaneuse, France.

mostafa.rabie@uni-saarland.de; jagadeesh.venki@gmail.com; Gertrud.Schmitt@uks.eu; henning.madry@uks.eu; veronique.migonney@wanadoo.fr; mmcucchiari@hotmail.com

Disclosures: None.

INTRODUCTION: Injuries of the anterior cruciate ligament (ACL), a key structure for knee stability, are highly prevalent and represent a major socioeconomic burden in orthopedics. Despite the availability of diverse therapeutic strategies including conservative management, surgical reconstruction with auto-/allografts, application of biologics, and use of synthetic scaffolds, none of them can fully restore the native ACL structure or replicate its original biomechanical properties. Gene therapy emerged as a promising alternative, offering sustained delivery and expression of therapeutic sequences directly within injured cells and tissues. Such approaches enable spatiotemporally controlled modulation of regenerative processes both *in vitro*, *ex vivo*, and *in vivo*, potentially overcoming the shortcomings of conventional therapies [1,2]. This work aimed at evaluating the therapeutic potential of recombinant adeno-associated virus (rAAV) vectors coding for the basic fibroblast growth factor (FGF-2) and the transforming growth factor beta (TGF- β) delivered via poly(sodium styrene sulfonate)-grafted poly(ϵ -caprolactone) (pNaSS-grafted PCL) films as a new platform to enhance the molecular and cellular mechanisms underlying ACL repair in a clinically relevant, experimental model of human ACL lesion *ex vivo* in conditions of experimental humoral neutralization reflecting the natural environment in patients *in vivo* [3,4].

METHODS: rAAV vectors (serotype 2) were packaged, purified, and titrated as previously described [5]. rAAV-*lacZ* carries the *E. coli* β -galactosidase (*lacZ*) reporter gene, rAAV-hFGF-2 a human FGF-2 sequence, and rAAV-hTGF- β a human transforming growth factor beta 1 sequence, all controlled by the CMV-IE promoter/enhancer [5]. Human ACL (hACL) explants were obtained from patients undergoing total knee arthroplasty at the Department of Orthopaedic Surgery, Homburg/Saar, Germany (n = 3/group; age range: 65-70 years). All samples were collected with informed consent and approval from the Ethics Committee of the Saarland Physician Council, in compliance with the Helsinki Declaration. The PCL films were prepared as previously described [5]. rAAV vectors encoding FGF-2, TGF- β , or *lacZ* gene were coated onto pNaSS-grafted PCL films (40 μ l per film, 8×10^5 transgene copies) [6,7]. Longitudinal lesions (5 mm length, 0.5 mm depth) were created in the explants and rAAV-coated PCL films were implanted directly in the defects. The study included eight experimental groups to evaluate the effects of rAAV vectors coated on pNaSS-grafted PCL films on ACL repair: (1) no vector, no PCL film (negative control); (2) no vector, with PCL film; (3) rAAV-*lacZ* (control vector), no PCL film; (4) rAAV-*lacZ*, with PCL film; (5) rAAV-hFGF-2, no PCL film; (6) rAAV-hFGF-2, with PCL film; (7) rAAV-hTGF- β , no PCL film; and (8) rAAV-hTGF- β , with PCL film. The explants were maintained for 21 days in DMEM supplemented with 10% FBS, 100 U/ml penicillin G, and 100 μ l/ml streptomycin in the presence of an anti-AAV capsid antibody (A20,1:200) (Progen) *ex vivo*. The culture medium was changed after the first 24 h and subsequently every two days until the end of the 21-day period. Transgene expression (FGF-2, TGF- β) was analyzed by ELISA (R&D Systems) [5]. The proteoglycan contents were monitored by binding to the dimethylmethylene blue dye and the DNA contents by Hoechst 33258 assay [5]. Total RNA was extracted using the RNeasy Protect Mini kit (Qiagen) and reverse transcription was carried out with the 1st Strand cDNA Synthesis kit (Qiagen). cDNA amplification was performed via SYBR Green RT-PCR [5]. Ct values were obtained for each target gene (type-I collagen - COL1A1, type-III collagen - COL3A1, decorin, tenascin, Mohawk, Scleraxis, IL-1 β , TNF- α) and for GAPDH as a control for normalization. Fold inductions (relative to control samples) were measured using the $2^{-\Delta\Delta Ct}$ method [5]. The one-way ANOVA followed by Tukey's multiple comparison tests was employed with $p < 0.05$ considered statistically significant.

RESULTS: Successful, efficient therapeutic delivery of rAAV-hFGF-2 and rAAV-hTGF- β via pNaSS-grafted PCL films was demonstrated in hACL lesions in the presence of the AAV neutralizing antibody, as seen by the sustained expression of FGF-2 (detectable on days 7, 14, and 21 for rAAV-hFGF-2) and TGF- β (detectable on days 14 and 21 for both treatments) compared with the controls (**Fig. 1**). Overexpression of rAAV-hTGF- β via pNaSS-grafted PCL films in the presence of the AAV neutralizing antibody modulated the biological activities of the explants after 21 days, resulting in a 13% increase in DNA content and a 10% decrease in the proteoglycan/DNA ratio relative to the negative controls (**Fig. 2**). Moreover, rAAV-hFGF-2 and rAAV-hTGF- β enhanced the deposition of extracellular matrix type-I and -III collagen, decorin, and tenascin-C (10-29%) and upregulated the expression of the tendon/ligament-specific transcription factors Mohawk and Scleraxis (10-20%) (**Fig. 3**). Importantly, these effects occurred without activating the expression of inflammatory mediators IL-1 β and TNF- α , indicating safe and effective therapeutic effects (**Fig. 3**).

DISCUSSION: The current study demonstrates the ability of rAAV-hFGF-2 and rAAV-hTGF- β delivered via pNaSS-grafted PCL films to enhance the reparative properties of experimentally injured human ACL in conditions mimicking natural gene transfer neutralization.

SIGNIFICANCE/CLINICAL RELEVANCE: pNaSS-grafted PCL film-guided rAAV-mediated gene transfer and overexpression of FGF-2 and TGF- β is a novel, potent strategy to promote the healing of hACL lesions in patients in future clinical applications in the naturally immune joint environment.

REFERENCES: [1] Madry *et al.*, *Am J Sports Med.* 2013, 41:194. [2] Amini *et al.*, *Int J Mol Sci.* 2023, 24:11140. [3] Abdul *et al.*, *Gene Ther.* 2023, 30:587. [4] Cottard *et al.*, *J Clin Immunol.* 2004, 24:162. [5] Stehle *et al.*, *Biotechnol Bioeng.* 2024, 121:3196. [6] Schrenker *et al.*, *Osteoarthritis Cartilage.* 2023, 31:467. [7] Venkatesan *et al.*, *Tissue Eng Part A.* 2020, 26:450.

ACKNOWLEDGEMENTS: Supported by a grant from the *Deutsche Forschungsgemeinschaft* (German Research Council, to MC, DFG CU 55/15-1).

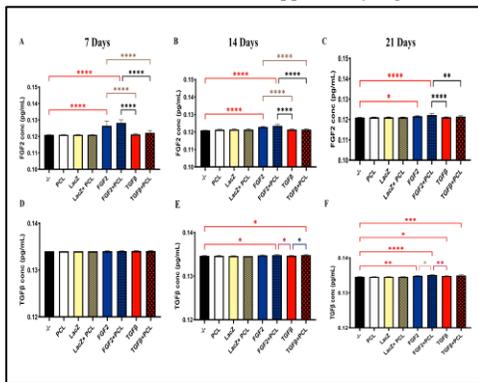


Figure 1. Detection of FGF-2 and TGF- β expression in hACL lesions treated with rAAV-hFGF-2 or rAAV-hTGF- β coated on pNaSS-grafted PCL films in the presence of A20 over time by ELISAs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

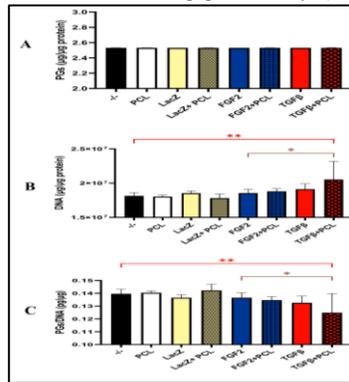


Figure 2. Biochemical analyses in hACL lesions treated with rAAV-hFGF-2 or rAAV-hTGF- β coated on pNaSS-grafted PCL films in the presence of A20 (day 21). * $p < 0.05$, ** $p < 0.01$.

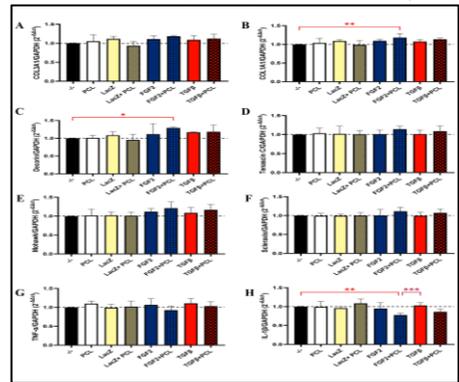


Figure 3. Gene expression profiles in hACL lesions treated with rAAV-hFGF-2 or rAAV-hTGF- β coated on pNaSS-grafted PCL films in the presence of A20 (day 21). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.