were narrowed in fundus examination, fundus images and fluorescein angiography, while in the other two transplants retinal vasculature seemed similar to the control eyes. There appeared to be decreased retinal perfusion in the animals with narrowed retinal arteries as compared to controls.

CONCLUSION: FA results have confirmed that retinochoroidal circulation can be established after WET in a rat model. Although 2 out of 4 rats exhibited some vascular attenuation in comparison to naïve rats, all rats exhibited a normal vascular filling pattern and the absence of vessel leakage which indicates that the structural integrity of blood-retinal barriers can be maintained after WET. The etiology of vascular attenuation and presumed decrease in retinal perfusion will be investigated in future studies.

Skin Wound Healing: The Effect of Cannabinoid CB1 Receptor Antagonism

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BACKGROUND: Fibroblasts are key players for maintaining skin homeostasis and for orchestrating physiological repair. They are present in all the phases of wound healing and their phenotype and function is changed under influence of various cytokines, of which transforming growth factor beta (TGF-β) stands out. TGF-β induces fibroblasts transdifferentiation into myofibroblasts and matrix preservation and deposition.1 CB1 receptor activation has been linked with fibrosis and formation of scar tissue in various tissues as in liver and skin.2-3 In fatty acid amide hydrolase knock-out mice it has been shown that elevated levels of endocannabinoids may induce skin fibrosis in a CB1 dependent manner.4 However, it is not known if cannabinoids have an effect in fibrinogenesis due to fibroblast activation or inflammation regulation. The aim of our study is to investigate whether CB1 ligands play a role in activation and differentiation of human fibroblasts.

MATERIAL AND METHODS: After informed consent, human skin samples were obtained from patients submitted abdominoplasty surgery. In total, samples from nine healthy patients with medium age of 37-years-old (23–51 years). Primary cultures of adult human fibroblasts were obtained from skin samples. Vimentin expression was used to confirm the presence of fibroblasts in-vitro. Human fibroblasts were stimulated with TGF-β (10ng/ml) to induce fibroblasts differentiation and then treated with CB1 ligands (AM251 10μM; ACEA 1μM). Trypan blue exclusion test for cell viability evaluation was performed. Fibroblasts activation into myofibroblasts was quantified by the expression of alpha smooth muscle actin (α-SMA) using Immunocytochemistry and Western Blotting assays. The significance of differences between means was assessed by Student’s unpaired t-test. Values of P < 0.05 are considered significant.

RESULTS: TGF-β induces fibroblast activation, measured by a 17-fold increase in the relative protein expression of α-SMA (mean ± SEM.: 17.00±6.1 in optical density). The CB1 agonist ACEA 1μM alone did not change (mean ± SEM.:1.1±0.2) fibroblasts activation neither affected differentiation induced by TGF-β (mean ± SEM.: 21.16±7.4). However, TGF-β effect was reverted in the presence of both CB1 agonist ACEA 1μM and CB1 antagonist AM251 10 μM (mean ± SEM.: 2.1±0.7). Moreover, the CB1 antagonist alone also reverts TGF-β effect (mean ± SEM: 2.2±0.1). Cell viability was not modified after treatment with CB1 agonists and antagonists or stimulation with TGF-β.

CONCLUSION: Inactivation of cannabinoid receptor CB1 reverts fibroblasts differentiation induced by TGF-β in human fibroblasts.

REFERENCES:
1. Finnson KW, McLean S, Di Guglielmo GM, Philip A. Dynamics of Transforming Growth Factor Beta Signaling in Wound Healing and Scarring. Adv Wound Care (New Rochelle). 2013;2(5):195–214.
2. Marquart S, Zerr P, Akhmetshina A, et al. Inactivation of the cannabinoid receptor CB1 prevents leukocyte infiltration and experimental fibrosis. Arthritis Rheum. 2010;62(11):3467–3476.
3. Dai E, Zhang J, Zhang D, et al. Rimonabant inhibits proliferation, collagen secretion and induces apoptosis in hepatic stellate cells. Hepatogastroenterology. 2014;61(135):2052–2061.
4. Palumbo-Zerr K, Horn A, Distler A, et al. Inactivation of fatty acid amide hydrolase exacerbates experimental fibrosis by enhanced endocannabinoid-mediated activation of CB1. Ann Rheum Dis. 2012;71(12):2051–2054.
Replacement of Contracted Split-Thickness Skin Graft and Keloid Scar with a Self-Propagating Autologous Skin Construct (SkinTE™)

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PURPOSE: The clinical standard of care for critically-sized cutaneous defects has remained meshed split-thickness skin grafts (STSGs), as seen in a variety of surgical, chronic, and burn wound reconstruction efforts. STSGs however, due to the intrinsic limited depth of harvest, remain unable to obtain the potent stem cell population which exists deeper within the dermis and therefore fail to regenerate cutaneous appendages, inter-follicular dermis as well as full-thickness skin. While there remain a variety of commercial skin substitutes which assist in healing cutaneous wounds, none have claimed to regenerate full thickness skin or appendages, until recently. SkinTE™ (PolarityTE, Salt Lake City, Utah), an autologous homologous human tissue product, has regenerated full-thickness skin in preclinical studies, and is further examined here in clinical application.

METHODS AND MATERIALS: A 10-year-old African American boy presented with chest, back, axillary, and elbow contractures, with interspersed keloid scars following flame burns two years prior. Following informed consent, a small sample of uninvolved full-thickness skin was harvested, three days later approximately 200 cm² of a previously scarred STSG across the left chest was excised, and the autologous SkinTE™ tissue product was applied intraoperatively. The SkinTE™ treated wound was then dressed and followed over the course of 6 weeks to determine the relative outcomes related to full-thickness skin regeneration, pigment development, relative contraction inhibition and scar reduction.

RESULTS: Full-thickness regeneration was noted beginning one week postoperatively, with rapid neo-dermal expansion, epithelialization and pigmentation occurring from discrete foci. Melanin deposition occurred throughout the wound with early return of pigmentation across the treated surface area. Additionally, contraction of the wound margin and peri-wound bed were minimal when comparing sequential images throughout the postoperative period. Macroscopic imaging of the interfaced margin of the wound (native tissue to SkinTE™) showed regions of complete scar resolution with minimal observed scar line.

CONCLUSION: Utilization of SkinTE appears to be a viable reconstructive technique for patients requiring full-thickness skin regeneration and/or replacement of the current clinical standard of care, a STSG. In this case, where a previously placed STSG with advanced scarring and keloid formation was resurfaced, the product appears to have regenerated full-thickness, pigmented skin with limited contraction.

Skin breakdown, infections, discomfort from scarring or heterotopic bone, and frequent adjustments due to residual limb volume changes limit the success of socket suspension technology for transfemoral (TF) amputees. These challenges prohibit many amputees from comfortably wearing their prosthesis or from being able to perform many activities. European and Australian programs have been implanting percutaneous osseointegrated (OI) devices in amputees for over two decades as an alternative means of artificial limb suspension that maintain a secure connection without the complications associated with socket technology. Reported risks of OI implants include mechanical failure, aseptic loosening and infection rates of 30–55%. No OI device has received full Premarket Approval by the FDA for clinical use within the United States. For the past ten years our research team has performed preclinical tests to design a new OI device, the Percutaneous Osseointegrated Prosthesis (POP), aiming to limit infection and provide a mechanically stable implant that allows for rapid return to ambulation. The FDA approved an