

## **Matrix therapy has new branch of regenerative medicine and its applications in burned treatment: From fundamental to clinic.**

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Matrix therapy a new branch of regenerative medicine and its applications in the treatment of burned: From basic to clinic.

Abstract:

RGTA®, for Regenerating Agents, form a new class of therapeutics. These molecules are polysaccharides substituted by functionalized groups selected to protect signal proteins called natural growth factors, cytokines, interleukins, chemokines,... against proteolytic degradation. These proteins play a key role in cellular communication and are naturally stored in the extracellular matrix via interactions with sulfated polysaccharides such as heparan sulfate. During tissue damage as burning, enzymes called heparanases degrade heparans sulfates, releasing these cytokines, which are then degraded. RGTA® could protect these cytokines or growth factors. This protection will extend their action and therefore their effectiveness. In the case of burns, this action would result in a modification of the collagen synthesis. This change allows a better repair of the lesion quickly visible on the appearance of tissues. This review presents the first experimental results of the use of RGTA® to treat burns in animal models by showing these actions at the molecular and histological level. Then a case of a human treatment with a medical device CACIPLIQ20® based on RGTA® technology is exposed in the treatment of a burn.

The RGTA® for Regenerating Agents form a new class of therapeutics that increases the speed and quality of tissue repair and in some cases, lead to a real tissue regeneration. The RGTA® are initially defined as polymers functionalized with carboxylic groups, sulfates and substitutions [1-2] promoting properties of penetration or anchoring in tissues (alkyl chains, lipids, aromatic) or other therapeutic agents (corticosteroids, antibiotics etc.).

They have the ability to mimic heparan sulfates, which are naturally present especially in the extracellular matrix and bind natural protein signals such as growth factors, cytokines, interleukins, etc... Their structure makes them resistant to proteolytic degradation [1]. They may as well as heparan sulfate ensure protection mission of different growth factors such as FGF-1 and -2 and TGF- $\beta$  even in heparanase rich environments such as tissue injury area [3]. They have already shown their regenerative capacity in various animal models but also in clinical trials [4-8].

During a tissue injury, of any kind (physical, chemical, viral, bacterial, ischemic...), a massive cell death occurs and the entire matrix architecture is destroyed. Heparan sulfates are rapidly degraded resulting in the destruction of growth factors that are no longer protected. Circulating and inflammatory cells are very quickly recruited at the site of injury and provide enzymes and growth factors that are totally different of those originally present in the tissue. Their action is to repair the injury as quickly as possible without attempting to comply with the local organization of cells. This action explains the appearance of a scar or fibrosis [9].

In case of burns, the activity increase of cytokines such as TGF- $\beta$  1 leads to change the network of collagen fibers with an overproduction of type III collagen [10-11] and then persistence of this expression for at least 10 months indicating long-term dysfunction in the healing process. The use of heparin in the treatment of burns is limited by the risk of adverse effects, including severe bleeding associated with anticoagulant activity, thrombocytopenia, or allergies [12-13]. The RGTA® nearly have no anticoagulant activity [14] and can therefore, by their action, restore the normal processes of collagen synthesis and avoid the appearance of scar while accelerating the process of tissue reconstruction.

Evolution of collagens during healing process.

The RGTA® OTR4120 was evaluated through matrix remodeling quality in an animal model of experimental skin burns in rats [15]. The burn was induced by a copper disk previously heated in boiling water for 5 seconds and applied on the dorsal skin of hairless rats. The wound was immediately flushed with saline or with a solution of 0.1 mg / ml OTR4120 diluted in saline buffer. The animals also received saline or OTR4120 (100mg/100g body weight) intramuscularly. Subsequently, the dermal application of saline or OTR4120 were repeated as before every three day during the first month then once a week for the next month. Intramuscular were repeated once a week for 3 months then once a month throughout the entire duration of the study namely 10 months after the burn. The animals were divided into 4 groups: healthy (unburned skin), healthy treated (unburned skin and treated by OTR4120), control (burned skin and treated with saline) and treated (burned skin and treated by OTR4120). Fibrotic index, which is the ratio of collagen III and collagen I, is an index to monitor quality of scar tissue. In the healthy group any action of RGTA is visible because lack of penetration of the product due to the absence of injury. During the healing of burned skin treated with saline, fibrotic index increases significantly compared to healthy skin during the first week after the induction of the burn. It remains high throughout the 10-month study (Figure 1). Another good marker in the evolution of wound healing was followed: matrix metalloproteinases (MMPs) which are a family of proteases involved in proteolytic degradation of many proteins of the extracellular matrix [16]. They can degrade all structural components of the extracellular matrix and thus the dermis, but also growth factors. Treatment with OTR4120 allows to keep an index close to that of healthy skin. (Figure 1). The abnormal increase in the synthesis of type III collagen observed in control animals is definitely not found when the animal is treated by OTR4120 (Figure 1). This specific effect of RGTA® on the type III collagen synthesis has already been described [17-18] and may involve an interaction with FGF-2 (fibroblast growth factors). It would be the result of better tissue reconstruction since the increased synthesis of type III collagen is often associated with fibrosis and excessive scarring. It has also been shown in previous studies that treatment with RGTA® decreases the production of type III collagen in the intestinal tissues of patients with Crohn's disease [19].

In this study, the expression of metalloproteinases MMP-2 and MMP-9 increased significantly in the burned skin, in agreement with data from human studies [10, 20]. They are key players in extracellular matrix remodeling such as the formation of vascular neointima. MMP-2, which is constitutively expressed, participates in the regulation of the constant degradation of collagen, whereas the expression of MMP-9 is induced during significant degradation of the extracellular matrix. OTR4120 can improve the activation of proMMP-2 and therefore the activity of MMP-2. Such as MMP-9 plays a crucial role in the remodeling of scar tissue [16], with his induction of a significant increasing of the both enzymes activity, OTR4120 may allow rapid and appropriate tissue remodeling.

Another possible explanation of the effects of OTR4120 lies in its ability to interact specifically with TGF- $\beta$ 1 (transforming growth factor) and improve its bioavailability [17, 21]. TGF- $\beta$ 1 is involved in the production and regeneration of the extracellular matrix under physiological conditions as in pathological conditions [22] and plays an essential role in control of fibrotic index by stimulating the production of type I collagen but also of type III collagen. In addition, TGF- $\beta$ 1 induces the production of MMP-2 and also stimulates the expression of MMP-9.

#### Histological changes during burns treatment

In the same model of thermal injury induced in rats, histological analysis of the burned skin treated or not by OTR4120 was conducted [23]. It appears that OTR4120 stimulates the production of new vessels from the early days. In addition, skin is more mature in the group treated with OTR4120 than in the control group, where three layers of granulomatous cells were visible, compared to four in the control group seven days after the burn. On each day during the first week, the stage of epidermal repair was approximately one day earlier in the RGTA group. In fact, the number of layers of keratinocytes is ever more important in the group treated with OTR4120 compared with the control group (Figure 2). Between 7 and 30 days, the epidermis is constantly thicker in the group OTR4120. However the quality of the newly formed epidermis appears to be similar in both groups. On day 14, the density of fibroblasts is higher in the group OTR4120. This effect could be attributed to the protective effect of OTR4120 on the FGF-2, which is chemotactic and mitogenic for fibroblasts *in vitro* and *in vivo*. The early development of a myofibroblastic appearance after treatment with OTR4120 can also be explained by the protection of FGF-2.

Keratin 14 was followed due to its properties as marker of keratinocyte division and epithelium during the skin restoration [24]. Indeed when the basal cells stop dividing and engage in the process of terminal differentiation that resulted in scales production, genes for keratin 5 and 14 are no longer expressed and are replaced by the expression of genes of keratin 1 and 10. In this model of burned skin, the presence of keratin 14 is greater in the group treated with OTR4120 than in the control group 3 days after burn induction. On day 4, the gene expression of keratin 14 peaked in the treated group larger than in the control group. On day 5, keratin 14 is found mainly in the epithelium in the OTR4120 group while it is confined to the granular layer and stratum spinosum in the control group. Thus, treatment with OTR4120 induces faster evolution kinetics of keratin 14 expression, in line with the histological elements showing an accelerated re-epithelialization.

Most of heparin binding growth factors play a key role in the healing of burns. Among them, VEGF (Vascular endothelial growth factor), a compound secreted by keratinocytes, macrophages, fibroblasts, have potent angiogenic effects and is related to the induction of endothelial cell division [25]. FGF-2 [26], also mediator of angiogenesis and epithelialization [27], accelerates the division of keratinocytes by stimulating the synthesis of matrix components such as collagen, fibronectin, and proteoglycans. It has been shown that topical application accelerates the healing of burns in a pig model [28] and enhances epithelialization of wounds in a diabetic mouse model [29]. In humans, topical application of FGF-2 on burns and chronic dermal ulcers can accelerate healing [30-32]. In a wound model induced in rats, administration clearly enhances the re-epithelialization and collagen synthesis by fibroblasts [33-35]. In humans, TGF- $\beta$  regulates angiogenesis and increases by actions on the synthesis of extracellular matrix, activation of fibroblasts and collagen synthesis and fibronectin production [36]. The OTR4120 seems to exert protective effects by maintaining the bioavailability of these growth factors. These factors are typically stored on heparan sulfates of the extracellular matrix but during a burn, the heparanases which are among the first enzymes activated, will destroy them and release growth factors that are then rapidly degraded. As the heparan sulfate mimetic OTR4120 can provide stable protection of these growth factors "heparin binding" [1], they

become less susceptible to be degraded despite the presence of heparanases. This protection allows a better action of growth factors in the reconstruction of the structure of the extracellular matrix and thus leads to a better repair of the injured area.

From laboratory to human applications:

RGTA® technology has resulted in several products that were developed in humans. Thus the first product, marketed as Class III medical device under the trade name of CACIPLIQ20® is indicated for the treatment of chronic skin wounds. A first clinical trial showed its effectiveness on peripheral arterial ulcers refractory to conventional treatment for average 7 months in patients with critical ischemia which could not or no longer have revascularization by vascular surgery. Two months of treatment led to the closure of half of the ulcers [37]. In another trial involving patients with pressure ulcers or venous ulcers towed for over two years, a reactivation of healing by CACIPLIQ20® was observed within the first month of treatment.

Relating to burns, the indication has not yet been documented. Case below is an ulceration caused by severe thermal injury in a neuropathic diabetic patient, stagnant for more than three months. Amputation was planned since gangrene began to set in, which is the classical evolution of this type of burn. Although CACIPLIQ20® is not indicated in the treatment of burns, its use allowed to offer an alternative. In accordance with its normal usage, CACIPLIQ20® was applied twice weekly using gauze, covering exposed areas visible on the picture (Figure 3). The gauze was left on the wound 5 minutes before being discarded. The wound was then covered with a bandage with a conventional non-adherent dressing like paraffin gauze dressing. The wound treated with RGTA® has restored almost entirely with a good quality skin after 4 months.

Several other patients with similar burns have been treated and cured without amputation. It was the same with acid burns or water boiling whose evolution was pessimistic. Thus the results seem to confirm on humans those obtained in animals models of burns. It highlights a possible extension for the use of CACIPLIQ20® in chronic or not burn wounds. Treatment by RGTA® or matrix therapy finds its place in brulology. Further investigations are needed to get better evaluation of its potential as therapy matrix used alone or in combination with matrix recovery system (for the protection of burned areas and exposed) or with cells from in vitro expansion or even grafts.

## Bibliography

1. M. Tardieu, C. Gamby, T. Avramoglou, Jozefonvicz J., and D. Barritault, Derivatized dextrans mimic heparin as stabilizers, potentiators, and protectors of acidic or basic FGF. *J Cell Physiol*, 1992. 150 (1): p. 194-203.
2. D. Ledoux, D. Papy-Garcia, Q. Escartin, MA Sagot, Y. Cao, et al., Human plasmin enzymatic activity is inhibited by Chemically modified dextrans. *J Biol Chem*, 2000. 275 (38): p. 29383-90.
3. Vlodavsky I., Friedmann Y., Elkin M., Aingorn H., Atzmon R., et al., Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. *Nat Med*, 1999. 5 (7): p. 793-802.
4. Zakine G., E. Martinod, P. Fornes, M. Sapoval, Barritault D., et al., Growth Factors Improve latissimus dorsi muscle vascularization and trophicity After cardiomyoplasty. *Ann Thorac Surg*, 2003. 75 (2): p. 549-54.
5. Blanquaert F., Saffar JL, Colombier ML, G. Carpentier, D. Barritault, and JP Caruelle, Heparan-like molecules Induce the repair of skull defects. *Bone*, 1995. 17 (6): p. 499-506.

6. Barbier-Chassefière V., Garcia-Filipe S., Yue XL, Kerros ME, Little E. et al., Matrix therapy in regenerative medicine, a new approach to chronic wound healing. *J Biomed Mater Res A*, 2009. 90 (3): p. 641-7.
7. Mr. Tong, Mr. Zbinden, Hekking IJ, M. Vermeij, Barritault D., and JW van Neck, RGTA OTR 4120 ®, heparan sulfate proteoglycan was mimetic, wound breaking strength and Increases vasodilatory capability in healing rat full-thickness excisional Wounds. *Wound Repair Regen* 2008. 16 (2): p. 294-9.
8. Chebbi CK, Kichenin K., N. Amar, H. Sepulveda, Warnet JM, et al. [Pilot study of a new matrix therapy agent (RGTA OTR4120 ®) in treatment-resistant corneal ulcers and corneal dystrophy]. *J Fr Ophthalmol*, 2008. 31 (5): p. 465-71.
9. Mutsaers SE, Bishop JE, McGrouther G., and Laurent GJ Mechanisms of tissue repair: from wound healing to fibrosis. *Int J Biochem Cell Biol*, 1997. 29 (1): p. 5-17.
10. Ulrich D, Noah EM, von Heimburg D., and N. PALLUA, TIMP-1, MMP-2, MMP-9, and PIIINP as serum markers for skin fibrosis in patients with severe burn trauma FOLLOWING. *Plast Reconstr Surg*, 2003. 111 (4): p. 1423-31.
11. Ulrich D, Noah EM, ER Burchardt, D. Atkins, and PALLUA, N., Serum concentration of amino-terminal propeptide of type III procollagen (PIIINP) as a prognostic marker for skin fibrosis scar correction in burned After patients. *Burns*, 2002. 28 (8): p. 766-71.
12. Jabr K., Johnson JH, McDonald MH, Walsh DL, Martin WD, AC Johnson, JM Pickett, and Shantha-Martin U., Plasma-modified ACT Can Be Used To monitor bivalirudin (Angiomax) anticoagulation for cardiopulmonary bypass on-pump surgery in a patient with heparin-induced thrombocytopenia. *J Extra Corpor Technol*, 2004. 36 (2): p. 174-7.
13. MJ Saliba Jr., Heparin in the Treatment of burns: a review. *Burns*, 2001. 27 (4): p. 349-58.
14. D. Papy-Garcia, V. Barbier-Chassefière, V. Rouet, Kerros M.-E., Klochendler C., et al., Nondegradative sulfation of polysaccharides. Synthesis and Structure Characterization of Biologically Active Heparan Sulfate Mimetics. *Macromolecules*, 2005. 38 (11): p. 4647 to 4654.
15. S. Garcia-Filipe, V. Barbier-Chassefière, C. Alexakis, E. Huet, D. Ledoux, et al., OTR4120 RGTA, a heparan sulfate mimetic, is a possible, long-term active agent to heal burned skin. *J Biomed Mater Res A*, 2007. 80 (1): p. 75 to 84.
16. G. Sawicki, Y. Marcoux, K. Sarkhosh, Tredget EE, Ghahary and A. Interaction of keratinocytes and fibroblasts modulates the Expression of matrix metalloproteinases-2 and -9 and Their inhibitors. *Mol Cell Biochem*, 2005. 269 (1-2): p. 209-16.
17. C. Alexakis, P. Mestries, S. Garcia, E. Petit, V. Barbier, et al., Structurally different RGTA ® s modulate collagen-type expression by cultured aortic smooth muscle cells via different pathways Involving fibroblast growth factor-2 gold transforming growth factor-beta1. *FASEB J*, 2004. 18 (10): p. 1147-9.
18. Mestries P., C. Borchellini, Barbaud C., A. Duchesnay, Escartin Q., et al., Chemically modified dextrans modulate expression of collagen phenotype of cultured smooth muscle cells by in relation to the degree of carboxymethyl, benzylamide, and sulfation substitutions . *J Biomed Mater Res*, 1998. 42 (2): p. 286-94.
19. C. Alexakis, Caruelle JP, Sezeur A., J. Cosnes, Gendre JP, et al., Reversal of abnormal collagen production in Crohn's disease intestinal biopsies treated fish with Regenerating agents. *Gut*, 2004. 53 (1): p. 85 to 90.
20. PK Young and F. Grinnell, Metalloproteinase Activation Cascade After Burn injury: a longitudinal analysis of the human wound environment. *J Invest Dermatol*, 1994. 103 (5): p. 660-4.
21. Mestries P., Alexakis C, Papy-Garcia D., A. Duchesnay, Barritault D., et al. Specific RGTA ® Increases collagen by cultured aortic term V smooth muscle cells via activation and protection of transforming growth factor-beta1. *Matrix Biol*, 2001. 20 (3): p. 171-81.

22. J. Thyberg, Differentiated properties and proliferation of arterial smooth muscle cells in culture. *Int Rev Cytol*, 1996. 169: p. 183-265.
23. Zakine G., V. Barbier, S. Garcia-Filipe, Luboinski J., Papy-Garcia D., et al., Matrix therapy with RGTA ® OTR4120 Improves healing time and quality in hairless rats with deep second-degree burns. *Plast Reconstr Surg*, 2011. 127 (2): p. 541-50.
24. Fuchs E. and H. Green, Changes in keratin gene expression DURING terminal differentiation of the keratinocyte. *Cell*, 1980. 19 (4): p. 1033-42.
25. V. Rouet, Y. Hamma-Kourbali, E. Petit, P. Panagopoulou, Katsoris P., et al., A synthetic glycosaminoglycan mimetic binds vascular endothelial growth factor and modulates angiogenesis. *J Biol Chem*, 2005. 280 (38): p. 32792-800.
26. Folkman J. and M. Klagsbrun, angiogenic factors. *Science*, 1987. 235 (4787): p. 442-7.
27. Hebda PA, Klingbeil CK, Abraham JA, Fiddes JC and, Basic fibroblast growth factor stimulation of epidermal wound healing in pigs. *J Invest Dermatol*, 1990. 95 (6): p. 626-31.
28. Danilenko DM, Ring BD, Tarpley JE, Morris B., Van G. Y., and al., Growth factors in porcine full and partial thickness burn repair: Differing targets and effects of keratinocyte growth factor, platelet-derived Growth Factor-BB, epidermal growth factor, and neu differentiation factor. *Am J Pathol*. 1995, 147:1261-1277.
29. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF Stimulate Wound Healing in the Genetically diabetic mouse. *Am J Pathol*. 1990, 136:1235-1246.
30. X. Fu, Z. Shen, Y. Chen, J. Xie, Z. Guo, Randomised placebo-controlled trial of use of topical recombinant bovine basic fibroblast growth factor for second-degree burns. *Lancet*, 1998. 352 (9141): p. 1661-4.
31. PA Jimenez and MA rampy, keratinocyte growth factor-2 ACCELERATES incisional wound healing in Wounds. *J Surg Res*, 1999. 81 (2): p. 238-42.
32. Staiano-Coico L, Krueger JG, Rubin JS, D'Limi S, Vallat VP, et al., Human keratinocyte growth factor effects in a porcine model of epidermal wound healing. *J Exp Med*, 1993. 178 (3): p. 865-78.
33. Soler AM, Wright TE, Smith PD, Maggi SP, Hill DP, et al., In vivo characterization of keratinocyte growth factor-2 as a potential wound healing agent. *Wound Repair Regen* 1999. 7 (3): p. 172-8.
34. Heldin CH and Westermark B. Platelet-derived growth factor: mechanism of actions and can function in vivo. *Cell Regul*, 1990. 1 (8): p. 555-66.
35. Brown RL, Breeden MP, Greenhalgh DG and, PDGF and TGF-alpha act synergistically to Improve wound healing in the diabetic mouse Genetically. *J Surg Res*, 1994. 56 (6): p. 562-70.
36. Sporn MB and Roberts AB, A major advance in the use of growth factors to Enhance wound healing. *J Clin Invest*, 1993. 92 (6): p. 2565-6.
37. P Desgranges, Louissaint T, Allaire E, Godeau B, K Kichenin, Becquemin JP, et al. Matrix therapy in vascular disease protection: first clinical pilot study of RGTA ® ®. Abstract published in the Proceedings of the International Meeting WHWS. Toronto, 2008.

Figure 1: Distribution of the synthesis of three types of collagen in the skin sample taken from mice that have not been burned (control) and burned (burned) with or without treatment with RGTA ®, 7 days after burn (top ) or 10 months after burn (bottom).

Figure 2: Histological Study on days 3 and 4 at x100 magnification. 3 days after the burn, the control group shows no layer of keratinocytes, a group OTR4120. On day 4, two layers are visible in the control group against five for the group treated with OTR4120.

Figure 3: Evolution of a burn patient treated with CACIPLIQ20 ® at the rate of application every 3 days. The evolution accomplished virtually complete regeneration of healthy skin in 4 months (125 days).