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Fragments of fibroblast growth factors useful in regenerative medicine components

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Introduction

Tissue engineering and/or regenerative medicine is a field of life science using different methods to create a new tissues/organs and/or to promote the regeneration of damaged or diseased tissues/organs. Materials used as scaffolds must ensure appropriate micro-environment of the cells, adhesion, proliferation, differentiation and finally the reproduction of the parent tissue [1,2]. Concentrated Growth Factors (CGFs) are the newest and safest therapy in regenerative medicine. CGF is a new-generation autologous platelet concentrate containing equivalent or higher levels of TGF-B1, PDGF-BB, and VEGF than platelet-rich plasma (PRP) concentrates. CGFs has been shown to stimulate cell proliferation [3,4], allowing for use in regenerative medicine. [5]. CGF is easily obtained by centrifuging intravenous blood of the patient and does not require any synthetics or biomaterials to make a gel; hence, it is free from the risk of cross-contamination [6]. The fibroblast growth factor (FGF) family represents a broad group of signaling proteins with structural similarities and related actions. FGFs are involved in a number of processes taking place in the cells; they are involved in wound regeneration, angiogenesis or regulation of metabolism, among others. The FGF family consists of 22 proteins. Apart from FGF15 and FGF19, the remaining FGFs have analogous mouse and human structures (homology about 90%). FGFs have very similar biological effects and a similar mechanism of gene expression Fibroblast growth factor 1 (FGF1) is also known as acidic fibroblast growth factor (aFGF). It consists of 155 amino acid residues, the main chain includes residues 16-155. FGF1 occurs as a monomer or homodimer. FGF1 binds to all receptors FGFR (FGFR1-FGFR4). In addition to homodimers, FGF1 forms a complex with fibroblast growth factor binding protein 1 (FGFBP1) and FGF2. Fibroblast growth factor 2 is also known as basic fibroblast growth factor (bFGF). The FGF2 chain consists of 288 amino acid residues, residues 1-142 constitute the propeptide. The main chain includes amino acids 143-288. It occurs as a monomer or homodimer. It binds to FGFR1, FGFR2, FGFR3 and FGFR4 receptors. FGF2 levels are increased in acute wounds, supporting the formation of granulation tissue, re-epithelialization and reconstruction of the damaged site [7]. It also regulates the synthesis and function of ECM components and increases the migration of keratinocytes and fibroblasts [8]. In the case of chronic wounds, the level of FGF2 is reduced [9]. Recombinant FGF2 applied directly to the wound shows promising effects in the treatment of pressure ulcers [10]. Promising effects have also been shown in the treatment of diabetic foot [11]. Currently, derivatives of fibroblast growth factors are available for the treatment of ulcers and burns. FGF proteins are recommended by the American Wound Healing Society and the European Wound Management Association for the treatment of refractory ulcers. Studies on the

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family of these proteins show that FGF2, FGF7 and FGF10 are key in the regeneration of skin wounds.

The aim of the study was to find a set of short fragments of native FGFs involved in repairing damaged tissues. These sets should reproduce the outer sphere of proteins and have biological properties similar to native proteins due to their ability to interact with receptors. We focused our attention on the search for FGF1 and FGF2 fragments.

Results and discussion

Using the SPOT method, libraries of FGF 1 and FGF2 fragments immobilized on cellulose were obtained [12]. Based on the Dot-blot test, fragments of both proteins were selected forming an outer sphere of proteins capable of interacting with the corresponding receptor.

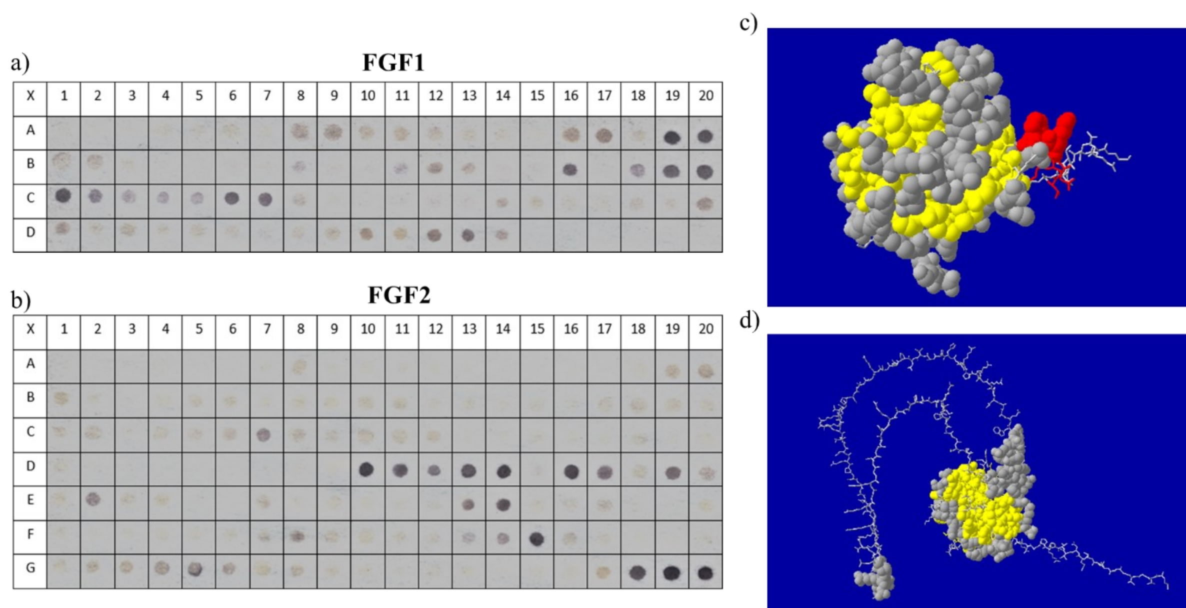

Fig. 1. a) Scan of a cellulose sheet containing the whole library of FGF1 fragments after reaction with antibodies. FGF1 map: 74 decapeptides, reading frame: 2 AA. b) Scan of a cellulose sheet containing the whole library of FGF2 fragments after reaction with antibodies. FGF2 map: 140 decapeptides, reading frame: 2 AA. c) FGF1 found fragments, AlphaFold model (Uniprot P05230). d) FGF2 found fragments, AlphaFold model (Uniprot P09038).

In the case of the FGF1 fragment library, 37 fragments able to interact with polyclonal antibodies were found based on the Dot-blot assay, including 17 fragments showing strong/very strong interaction. For the FGF2 fragment library, 20 fragments able to interact with polyclonal antibodies were found, of which 14 fragments showed strong/very strong interaction. The found fragments of FGF1 and FGF2 were synthesized on 2-chlorochlorotrityl resin using a triazine coupling reagent [13]. In the next step, FGF fragments were used in studies on NIH/3T3 cells to investigate their effect on the viability of cells incubated in a medium with the addition of FGF fragments.

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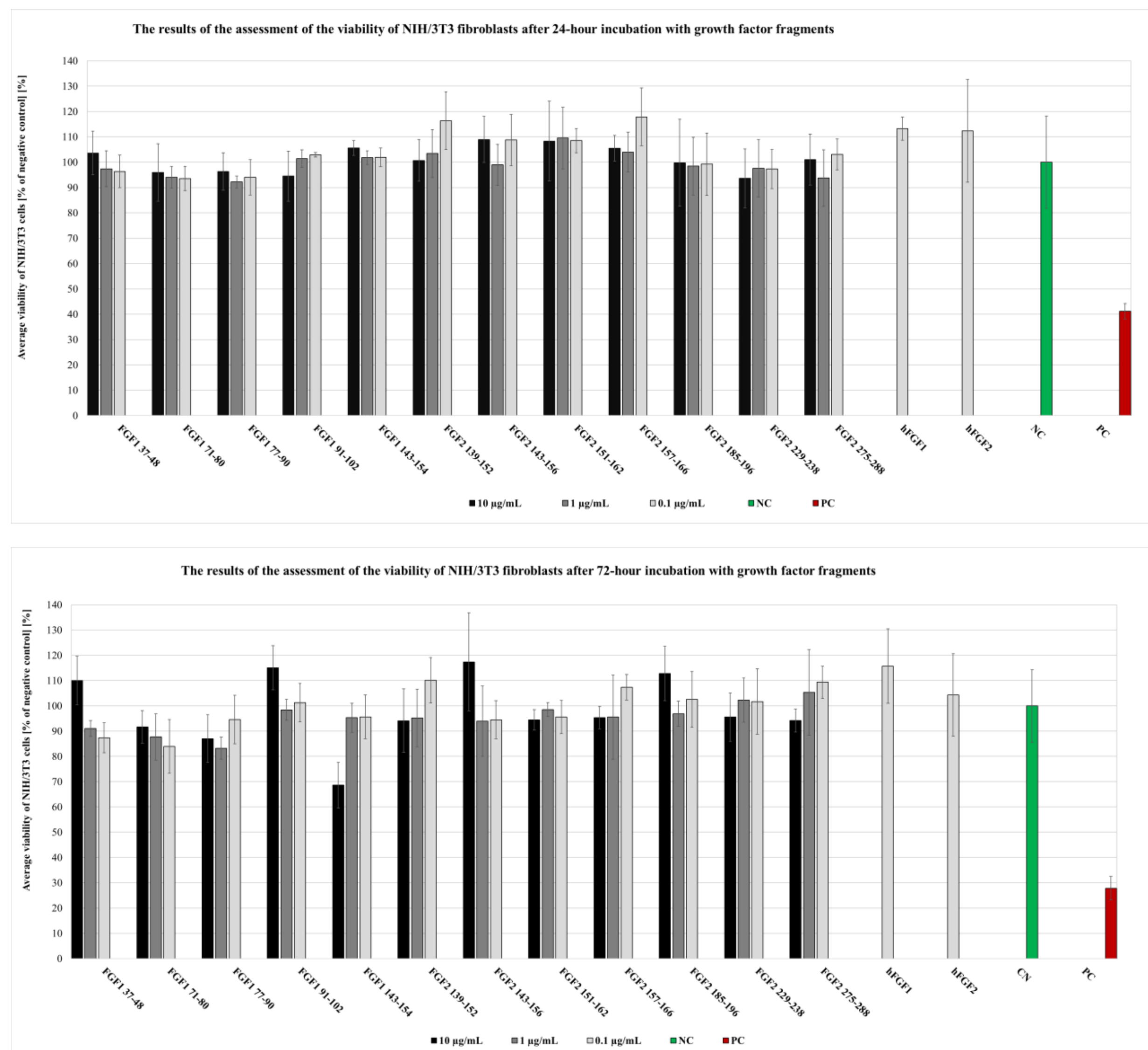

Fig. 2 Viability of NIH/3T3 cells grown in the presence of FGF1 and FGF2 fragments. Incubation for 24 h (top panel), incubation for 72 h (bottom panel).

Studies have shown that all FGF1 and FGF2 fragments have no negative effect on cell viability at concentrations of 1 and 0.1 µg/mL. However, at a concentration of 10 µg/mL, only one FGF1 fragment (cultured for 72 hours) showed a slight decrease in cell viability.

Conclusions

Selected FGFs fragments are useful in the regeneration of skin damage and treatment of difficult-to-heal wounds.

Literature

[1] Wobma, H., Vunjak-Novakovic, G. (2016) Tissue Engineering and Regenerative Medicine 2015: A Year in Review. *Tissue Eng. Part B Rev. 22*, 101-113. doi: 10.1089/ten.TEB.2015.0535.
[2] Marx, V. (2015) Organs from the lab *Nature 522*, 373-377. doi.org/10.1038/522373a.
[3] Masuki, H., Okudera, T., Watanebe, T., Suzuki, M., Nishiyama, K., Okudera, H. (2016) Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *Int. J. Implant Dent. 2*, 19. doi.org/10.1186/s40729-016-0052-4.
[4] Giannotti, L., Di Chiara Stanca, B., Spedicato, F., Nitti, P., Damiano, F., Demitri, C., Calabriso, N., Carluccio, M.A., Palermo, A., Siculella, L. (2023) Progress in Regenerative Medicine: Exploring Autologous Platelet Concentrates and Their Clinical Applications. *Genes 14*, 1669. doi.org/10.3390/genes14091669.

[5] Yahata, Y., Handa, K., Ohkura, N., Okamoto, M., Ohshima, J., Itoh, S., Kawashima, N., Tanaka, T., Sato, N., Noiri, Y., Hayashi, M., Okiji, T., Saito, M. (2023) Autologous concentrated growth factor mediated accelerated bone healing in root-end microsurgery: A multicenter randomized clinical trial. *Regen*. *Ther. 24*, 377-384. doi.org/10.1016/j.reth.2023.08.006.
[6] Rodella, L.F., Favero, G., Boninsegna, R., Buffoli, B., Labanca, M., Scarì, G., Sacco, L., Batani, T., Rezzani, R. (2011) Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microsc. Res. Tech. 74*, 772-777. doi: 10.1002/jemt.20968.
[7] Powers, C.J., McLeskey, S.W., Wellstein. A. (2000) Fibroblast growth factors, their receptors and signaling. *Endocr. Relat. Cancer 7*, 165-197. doi:10.1677/erc.0.0070165.
[8] Sogabe, Y., Abe, M., Yokoyama, Y., Ishikawa, O. (2006)Basic fibroblast growth factor stimulates human keratinocyte motility by Rac activation. *Wound Repair Regen*. *14*, 457-462. doi:10.1111/j.1743-6109.2006.00143.x
[9] Robson, M.C. (1997)The role of growth factors in the healing of chronic wounds. *Wound Repair Regen*. *5*, 12-17. doi:10.1046/j.1524-475X.1997.50106.x
[10] Payne, W.G., Ochs, D.E., Meltzer, D.D., Hill, D.P., Mannari, R.J., Robson, L.E., Robson, M.C (2001) Long-term outcome study of growth factor-treated pressure ulcers. *Am J Surg*. *181*, 81-86. doi:10.1016/S0002-9610(00)00536-5.
[11] Uchi, H., Igarashi, A., Urabe, K., Koga, T., Nakayama, J., Kawamori, R., Tamaki, K., Hirakata, H., Ohura, T., Furue, M. (2009) Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer. *Eur. J. Dermatology*. *19*, 461-468. doi:10.1684/ejd.2009.0750.
[12] Fraczyk, J., Walczak, M., Kaminski, Z.J. (2018) New methodology for automated SPOT synthesis of peptides on cellulose using 1,3,5-triazine derivatives as linkers and as coupling reagents. *J. Pept. Sci.*, *24*, e3063. doi.org/10.1002/psc.3063.
[13] Kolesinska, B., Rozniakowski, K.K., Fraczyk, J., Relich, I., Papini, A.M., Kaminski, Z.J. (2015) The Effect of Counterion and Tertiary Amine on the Efficiency of N-Triazinylammonium Sulfonates in Solution and Solid-Phase Peptide Synthesis. *Eur. J. Org. Chem*., 401-408. doi: 10.1002/ejoc.201402862.