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B7-33 LOADED DRESSINGS: NOVEL APPROACH FOR MANAGING HYPERTROPHIC SCARS FROM BURN INJURIES

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Riassunto  
Severe burns can result in hypertrophic scars (HTS) that significantly impact a patient's quality of life both physically and psychologically [1]. Once HTS forms, traditional treatments are often minimally effective, and currently, there is a lack of preventive treatments for HTS. Therefore, there is a growing focus on developing anti-fibrotic formulations to manage HTS [2]. B7-33, a synthetic single-B-chain analogue of human relaxin-2 peptide (H2-RLX), has demonstrated effectiveness as an anti-fibrotic agent in various fibrotic conditions [3]. However, there have been no studies exploring the potential of B7-33 peptide against HTS. The objective of this study was to develop an advanced dressing incorporating B7-33 as a potential treatment for HTS resulting from burn injuries.

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The cytotoxicity of the B7-33 peptide was evaluated using the MTT assay on Human Hypertrophic Scar Fibroblasts (HSFs) and confirmed with Live/Dead stain. Its impact on fibrotic targets, including Transforming Growth Factor  $\beta$ 1 (TGF- $\beta$ 1), Collagen-Type1-alpha1 chain (COL1A1), and  $\alpha$ -Smooth Muscle Actin ( $\alpha$ -SMA), was assessed through gene expression analyses. Additionally, the ability of B7-33 to modulate the expression of fibrotic targets was compared with Pirfenidone (PF), a recognized antifibrotic agent [4]. Following this, B7-33 was incorporated into poly-lactide-co-poly- $\epsilon$ -caprolactone (PLA-PCL) and poly-lactic-co-glycolic acid (PLGA) advanced dressings as an efficient drug delivery system. The formulations were characterized in vitro for their physical properties, morphology, and drug release behaviour.

B7-33 peptide has been shown to be non-toxic, with cell viability ranging from  $86.87 \pm 2.64\%$  to  $100 \pm 11.80\%$  after 24, 48 and 72h. Live/Dead assays showed spindle-shaped cells with no evidence of damage or apoptosis. Gene expression analysis on HSF showed the ability of B7-33 downregulate fibrotic targets within 72h and at a lower concentration than PF. Morphological analysis by SEM and AFM revealed randomly distributed, homogeneous fibers without peptide crystals or residues on fibre surface. Mean fibre diameter was  $2.82 \pm 0.24 \mu\text{m}$ , size distribution ranged from 8.0nm to 100  $\mu\text{m}$ , and mean pore size was  $1171.92 \pm 151.81 \mu\text{m}^2$ . In-vitro release profile showed  $11 \pm 1.5\%$  release in the first 6 hours, followed by gradual release over 168 hours.

The results were promising, and ongoing studies are underway to further assess the biological effectiveness of B7-33 dressings on HTS.

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[2] Tottoli, E.M.; Dorati, R.; Genta, I.; Chiesa, E.; Pisani, S.; Conti, B. Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. *Pharmaceutics* **2020**, *12*, 735.

[3] Praveen, P.; Wang, C.; Handley, T.N.G.; Wu, H.; Samuel, C.S.; Bathgate, R.A.D.; Hossain, M.A. A Lipidated Single-B-Chain Derivative of Relaxin Exhibits Improved In Vitro Serum Stability without Altering Activity. *Int. J. Mol. Sci.* **2023**, *24*, 6616.

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