PDGF-Derived Peptides: From Receptor Binding to Cellular Signaling in Wound Healing

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Introduction

Clinical background

Chronic wounds affect 1-2% of Europeans annually with, treatment costs reaching 2-3% of healthcare budgets.

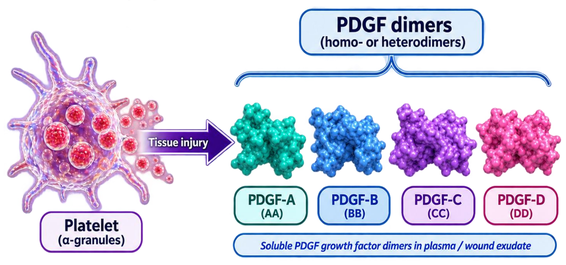


Patients with diabetes are particularly vunlnerable to diabetic foot ulcers and other chronić wounds.

The underlying pathophysiology involves i.e. impaired tissue repair, neuropathy and deficiency of growth factors, notably PDGF (Platelet-Derived Growth Factor) and VEGF (Vascular Endothelial Growth Factor) [1].

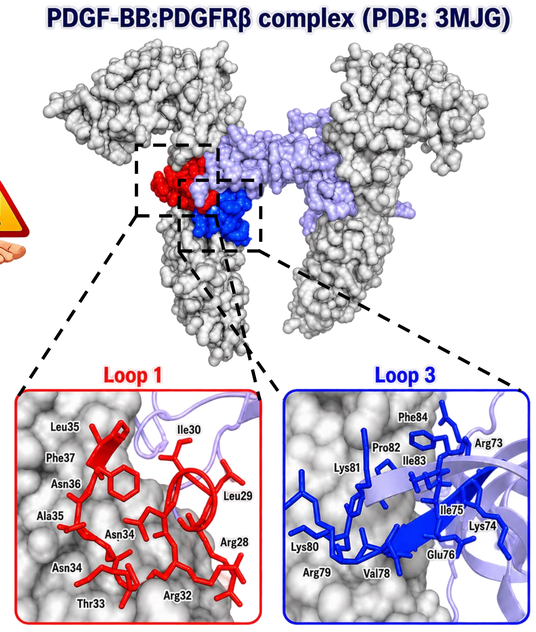
PDGF biology

PDGF proteins are stored in platelet α-granules and released after tissue injury as homo- or heterodimers.



Among these isoforms, PDGF-BB plays a central role in wound healing from injury to scar formation.

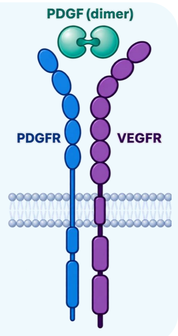
PDGF-BB:PDGFRβ complex (PDB: 3MJG)



- Activation of PDGFR and VEGFR receptors requires the binding of the protein in dimeric form, which induces autophosphorylation and the initiation of signalling cascades [2].
- The binding of PDGF and VEGF proteins to the receptor is mediated by so-called binding loops.

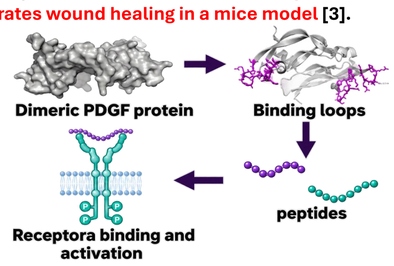
PDGF cross-activity

- PDGF and VEGF proteins show structural similarity, resulting in partial cross-activity between their receptors.
- Several PDGF (PDGF-AA, PDGF-BB, PDGF-CC) isoforms have been shown to bind VEGFR2. This structural and functional overlap suggests that PDGF-derived peptides may retain or acquire a broader receptor-binding profile.



Our research

Our team's research on the PDGF-BB protein has led to the identification of a fragment (the L1 loop region) that effectively stimulates skin cell proliferation and accelerates wound healing in a mice model [3].



Encouraged by our previous studies on the pro-regenerative potential of binding loops derived from the PDGF-BB protein, we decided to investigate the remaining isoforms of this protein, namely PDGF-A, PDGF-C, and PDGF-D.

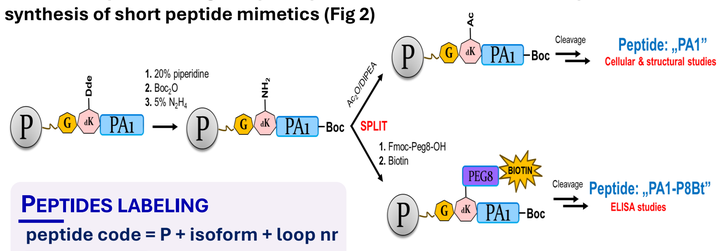
Peptide design and synthesis

Based on the amino acid sequences of the binding loop regions, aligned according to the conserved cystine knot motif, we designed (*Fig. 1*) a series of novel peptides.

Protein:	Peptide:		Sequence		Loop
hPDGF-A	PA1	94	:AVCK-TRTVIYEIPRSQVDPTSANFLIWPPCVEVKRCT---GCC-----NT:	135	Loop„L1"
hPDGF-B	PB1	95	:AECK-TRTEVFEISHRLIDRTNANFLIWPPCVEVQRCS---GCC-----NN:	136	
hPDGF-C	PC1	248	:YSCT-PRNFSVSIRELK---RTDTIFWPPCLLVKRCG---GNCACCLHNC:	291	
hPDGF-D	PD1	270	:YSCT-PRNYSVNIRELK---LANVVFFPRCLLVQRCG---GNCGCGTVNW:	313	
hPDGF-A	PA3	136	:SSVKCQPSRVHHRSVKVAKVEYVRKKPKLK-----EVQVRLEEHLECACAT:	181	Loop„L3"
hPDGF-B	PB3	137	:RNVQCRPTQVQLRPVQVRKIEIVRKKPIFK-----KATVTLEDHLACKCET:	182	
hPDGF-C	PC3	292	:NECQCVPSKVTKKYHEVLQLRP---KTGVRGLHKSLTDVALEHHEECDCVC:	339	
hPDGF-D	PD3	314	:RSCTCNSGKTVKKYHEVLQFEPGHIKRRGRAKTMALVDIQLDHHERCDCTC:	364	

Fig. 1 Sequences of peptides derived from L1 and L3 loops of PDGFs.

PDGF receptor-binding loop sequences were used as templates for the synthesis of short peptide mimetics (Fig 2)



Peptides labeling

peptide code = P + isoform + loop nr

P = PDGF	Loops:
isoforms A-D	Loop 1 & Loop 3

PA1 = P A 1
e.g. label PDGF isoform loop

Fig. 2 Schematic flow of peptides' synthesis.

Methodology

1. Screening and initial characterization

ELISA-based affinity screening: lack 96-well plates were Ni^{2+}-functionalized to immobilize 6×His-tagged **PDGFRα, PDGFRβ, VEGFR1 and VEGFR2**. **Biotinylated peptides** were added, and receptor binding was detected using **streptavidin–alkaline phosphatase** and **AttoPhos** substrate. Fluorescence was measured kinetically using a Tecan Infinite 200 plate reader.

Cell-based assays: Human **HaCaT keratinocytes, 46BR.1N and BJ fibroblasts** were cultured in DMEM supplemented with 10% FBS at 37°C and 5% CO_2. Cells were serum-starved for 24 h prior to peptide treatment. **Proliferation** was assessed using the XTT assay after 48 and 72 h of stimulation, while **cytotoxicity** was evaluated by measuring LDH release into supernatants after 48 h.

2.Detailed characterization of selected peptides

Microscale thermophoresis: Receptor proteins were fluorescently labeled using the **RED-NHS 2nd Generation** labeling kit (NanoTemper). Peptides were dissolved in water and serially diluted in **50 mM Tris buffer, pH 8.0, containing 0.05% Pluronic**. Measurements were performed in **spectral shift mode at 650 and 670 nm** using a Monolith instrument (NanoTemper).

Serum stability: Peptides at a concentration of **1 mM** were incubated with human serum in a **1:1 ratio**. Samples were collected at defined time points, and serum proteins were precipitated with **15% TCA** followed by centrifugation. Peptide degradation was monitored chromatographically based on changes in peak area. **Mass spectra were recorded for samples containing approximately 50% of the initial peptide amount** to identify degradation products

Circular Dichroism: Peptide structure was analyzed in the **190–260 nm range** using a Jasco J-815 spectropolarimeter, with **buffer-subtracted** spectra reported as mean residue ellipticity.

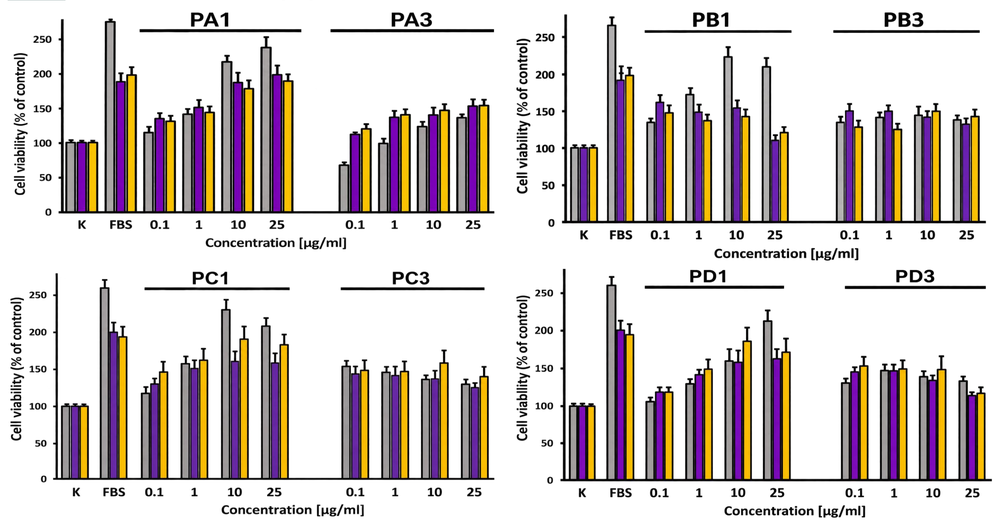
Screening Phase

One peptide library • two complementary biological readouts

8 PDGF-Derived peptides

PDGF-A •PDGF-B • PDGF-C • PDGF-D | Loop 1 + Loop 3

Cellular studies of PDGF peptides



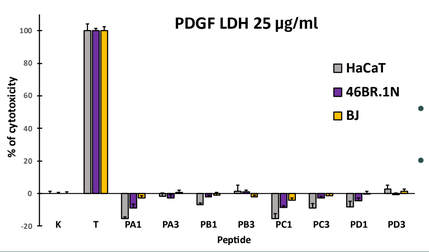
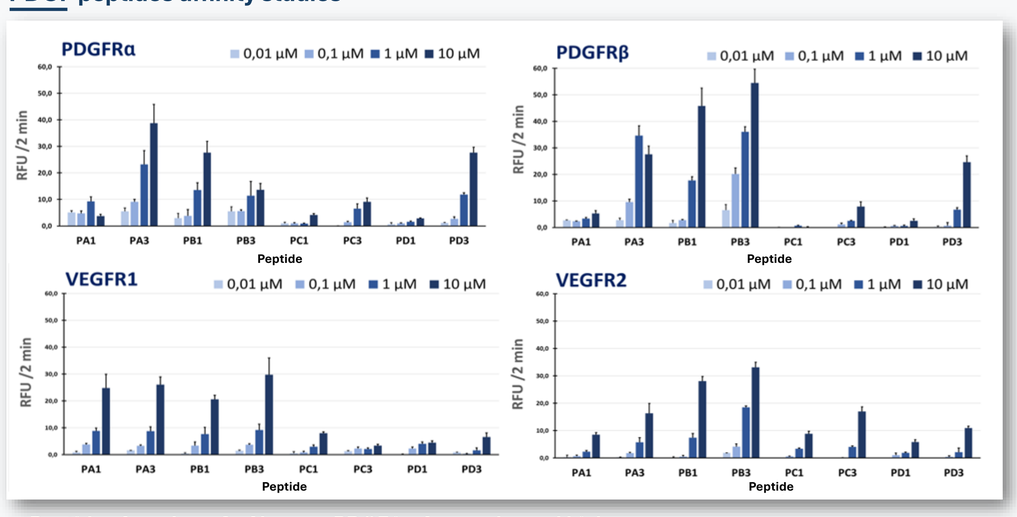


Fig. 3 Proliferation of HaCaT, 46Br.1N and BJ cells after 72h incubation with PDGF-derrived peptides.

- All PDGF peptides stimulated cel proliferation, whereas peptides designed from the L1 loop show stronger effects.
- The pro-proliferative effect is most strongly visible in keratinocytes.

Fig. 4 Cytotoxicity of PDGF-derrived peptides

PDGF peptides affinity studies



- Peptides from loop 3 of human PDGF isoforms showed higher affinity for receptors,
- Peptides from isoforms A and B exhibiting the strongest affinity.

Fig. 5 Affinity of the PDGF peptides to receptors from PDGFR and VEGFR groups

KEY SCREENING CONTRAST

LOOP 1 stronger cellular response

LOOP 3 stronger receptor binding

Selected for detailed characterization

Integrating cellular activity + receptor binding behavior

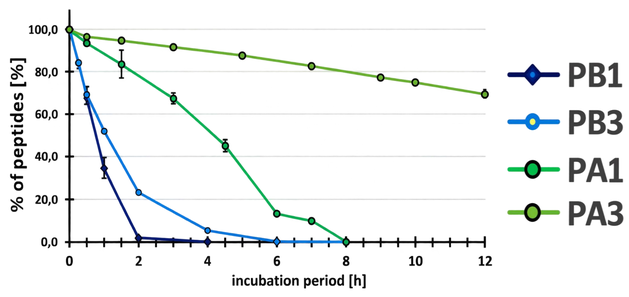
PA1 PA3 PB1 PB3

Detailed Characterization

A focused „deep dive" into four lead peptides selected from the screening phase

Serum stability

Percentage change in the quantity of PDGF peptides over time



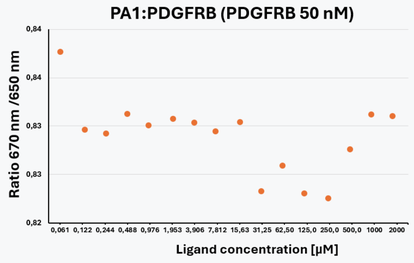
Identified cleavage sites and peptide half-lives:

		$t_{1/2}$
PB1	R-L-I-DRTNANFLk(Ac)G-NH_2	40 min
PB3	R-K-I-EIVRKKPIFk(Ac)G-NH_2	1 h
PA1	S-Q-V-D-PTSANFLk(Ac)G-NH_2	4,5 h
PA3	A-K-VEYVRKKPKLk(Ac)G-NH_2	-

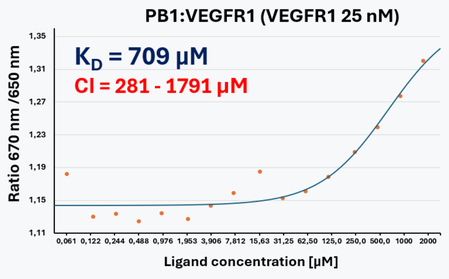
Microscale thermophoresis

Types of curves recorded during MST analysis:

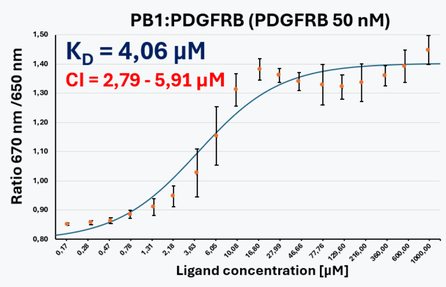
a) No binding



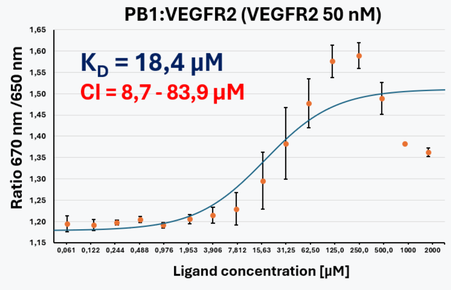
b) No plateau



c) A correct curve with the K_D value plotted



d) Non-standard curve shape



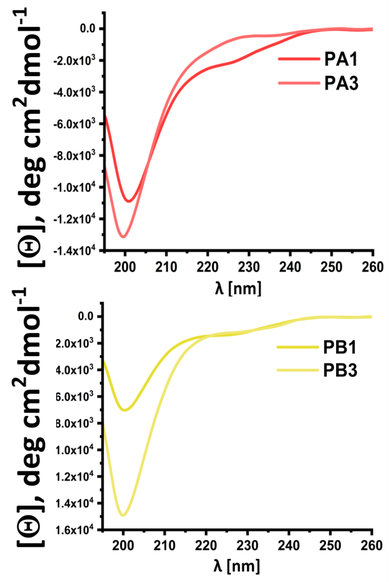
Dissociation constants K_D:

	PDGFRα	PDGFRβ
PA1	2,09 mM (1,64-2,67)	-
PA3	28,5 µM (22,2-36,4)	8,22 µM (5,61-12,02)
PB1	20,8 µM (15-28,8)	4,06 µM (2,79-5,91)
PB3	39,3 µM (31,3-49,4)	9,12 µM (7,01-11,86)

	VEGFR1	VEGFR2
PA1	-	-
PA3	-	1,55 µM (0,87-2,79)
PB1	709 µM (281-1791)	18,4 µM (8,7-83,9)
PB3	354 nM (86-1464)	1,39 µM (0,99-1,95)

Circular dichroism spectra

Peptides have a disordered structure typical of short peptides;



Conclusions

- PDGF-derived peptides were non-cytoxic and stimulated proliferation of skin-derived cells, with Loop 1 peptides showing stronger pro-proliferative effects.
- ELISA and MST confirmed interactions of selected peptides; however, stronger receptor binding did not directly translate into higer cellular activity.
- MST reveald non-classical binding profiles for some peptide-receptor pairs, suggesting more complex interaction modes.
- Serum stability was strongly sequence-dependent, while MS analysis indicated predominantly N-terminal peptide degradation.

Literature:

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[3] M. Deptuła, P. Karpowicz, A.Wardowska et al., *Adv. Wound Care*, 2020, vol. 9, no. 12, pp. 657-675

National Science Centre

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