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The angiogenic response to PLL-g-PEG-mediated HIF-1α plasmid DNA delivery in healthy and diabetic rats

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ABSTRACT

Impaired angiogenesis is a major clinical problem and affects wound healing especially in diabetic patients. Improving angiogenesis is a reasonable strategy to increase diabetes-impaired wound healing. Recently, our lab described a system of transient gene expression due to pegylated poly-L-lysine (PLL-g-PEG) polymer-mediated plasmid DNA delivery *in vitro*. Here we synthesized peptide-modified PLL-g-PEG polymers with two functionalities, characterized them *in vitro* and utilized them *in vivo* via a fibrin-based delivery matrix to induce dermal wound angiogenesis in diabetic rats. The two peptides were 1) a TG-peptide to covalently bind these nanocondensates to the fibrin matrix (TG-peptide) for a sustained release and 2) a polyR peptide to improve cellular uptake of these nanocondensates. In order to induce angiogenesis *in vivo* we condensed modified and non-modified polymers with plasmid DNA encoding a truncated form of the therapeutic candidate gene hypoxia-inducible transcription factor 1α (HIF-1α). HIF-1α is the primarily oxygen-dependent regulated subunit of the heterodimeric transcription factor HIF-1, which controls angiogenesis among other physiological pathways. The truncated form of HIF-1α lacks the oxygen-dependent degradation domain (ODD) and therefore escapes degradation under normoxic conditions. PLL-g-PEG polymer-mediated HIF-1α–ΔODD plasmid DNA delivery was found to lead to a transiently induced gene expression of angiogenesis-related genes Acta2 and Pecam1 as well as the HIF-1α target gene *Vegf in vivo*. Furthermore, HIF-1α gene delivery was shown to enhance the number endothelial cells and smooth muscle cells – precursors for mature blood vessels – during wound healing. We show that – depending on the selection of the therapeutic target gene – PLL-g-PEG nanocondensates are a promising alternative to viral DNA delivery approaches, which might pose a risk to health.

1. Introduction

Wound healing is a highly dynamic process comprising several overlapping stages: haemostasis/inflammation, migration, proliferation and maturation phases. It involves complex interactions of extracellular (ECM) molecules, soluble mediators, various resident cells and infiltrating cells to achieve the goal of tissue integrity [1]. Diseases such as diabetes mellitus interfere with wound healing by disrupting the orderly sequence of events at one or more of the stages, thereby compromising the wound-healing process. The deficiency of endogenous growth factors [2,3] and/or the excessive production of exudates and expression of high levels of tissue destructive proteinases [4] are associated to chronic wound formation. Diabetic ulcerations are characterized by impaired neovascularization and blood perfusion. Approximately 5–15% of diabetes patients are prone to foot ulcer development [5], requiring an appropriate strategy to improve wound healing.

Gene therapy has gained increasing attention as an alternative approach for wound treatment [6–8]. Especially non-viral gene transfer benefits from biosafety and the unlimited gene size

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transportation capacity [9]. However, the major drawbacks of non-viral vectors are their poor *in vivo* transfection efficiencies resulting in low protein production, as well as their transient gene expression profile, which for improvement of local and temporal wound healing, is desirable. Therapeutic DNA is able to condense with poly-cationic substances such as poly-L-lysine (PLL), poly-L-ornithin or polyethylenimine (PEI) [10–13]. Polymers with high cationic density confer cytotoxicity [14], which is circumvented by the formation of different block-copolymers between poly(ethylene glycol) (PEG) and PLL, PEI or poly-aspartic acid [10,13–15]. Polymer-DNA condensates using grafted copolymers of PLL and PEG have been shown to combine low cytotoxicity, stealth properties and high transfection efficiency in COS-7 cells [16,17], suggesting that they are a promising tool for effective transport and delivery of therapeutic DNA.

An excellent candidate protein to support wound revascularization is the transcription factor hypoxia-inducible factor 1 (HIF-1). Among other physiological pathways, HIF-1 controls angiogenesis by regulating the expression of pro-angiogenic target genes including *Vegf-a* [18]. HIF-1 is an oxygen-responsive, heterodimeric transcription factor consisting of an alpha-subunit and a beta-subunit. In order to quickly react to changes in oxygen concentrations, both subunits are constitutively expressed with HIF-1α being posttranslationally regulated by oxygen availability. Prolylhydroxylases (PHDs) hydroxylate HIF-1α at two specific proline residues that are located in the oxygen-dependent degradation domain (ODD) [19–21] in an oxygen-dependent manner. This targets HIF-1α for the binding of the tumor suppressor von-Hippel–Lindau protein (VHL), which initiates the accumulative binding of ubiquitin and eventually leads to proteasomal degradation of HIF-1α [22]. Additionally, factor inhibiting HIF (FIH) hydroxylates human HIF-1α subunits at an asparagine residue in the C-terminal transactivation domain. This interferes with the binding of the essential transcriptional co-activators p300 and CBP (CREB binding protein) and reduces the transcriptional activity of HIF-1 [23]. An engineered variant of HIF-1α, which lacks the ODD, escapes the oxygen-dependent degradation – resulting in stable expression under normoxic conditions. Recently, condensates of HIF-1α–ΔODD plasmid DNA and peptides were shown to increase the number and the quality of newly formed blood vessels in full-thickness excision dermal wounds of healthy mice after release from modified 3D fibrin matrices [24]. Fibrin hydrogels harbor a great wound healing potential. They are among the most often used native hydrogels to induce angiogenesis and wound healing *per se* [25,26] and have been widely used as a drug delivery system [27,28].

Since the formation of new blood vessels is a prerequisite for successful wound healing, we tested whether PLL-g-PEG based polymers deliver potentially therapeutic HIF-1α–ΔODD plasmid DNA and induce blood vessel formation *in vivo*. Thus, we produced and employed 3D fibrin matrices as release system for the local gene therapeutic approach and analyzed the effect of HIF-1α–ΔODD plasmid DNA delivery on wound revascularization under normal and diabetic conditions *in vivo*.

2. Materials and methods

2.1. Nomenclature of PLL-g-PEG polymers

Three different PLL-g-PEG polymers were used in this study (Fig. 1) consisting of a 20 kDa poly-L-lysine (PLL) backbone grafted with 5 kDa poly(ethylene glycol) (PEG). Grafting density (g) indicates the percentage of pegylated lysine residues. TG and polyR abbreviate peptide sequences encoding either a transglutaminase recognition sequence allowing covalent incorporation of the polymer into fibrin

A

Nomenclature	PLL (kDa)	PEG (kDa)	PEG grafting density g [%]	Functionalizing Peptide
PLL_{20}-g_5-PEG_5	20 kDa	5 kDa	5 %	
PLL_{20}-g_8-PEG_5-TG	20 kDa	5 kDa	8 %	**TG**: NQEQVSPLGYERCG
PLL_{20}-g_3-PEG_5-polyR	20 kDa	5 kDa	3 %	**polyR**: RRRRRRRGYGRRC

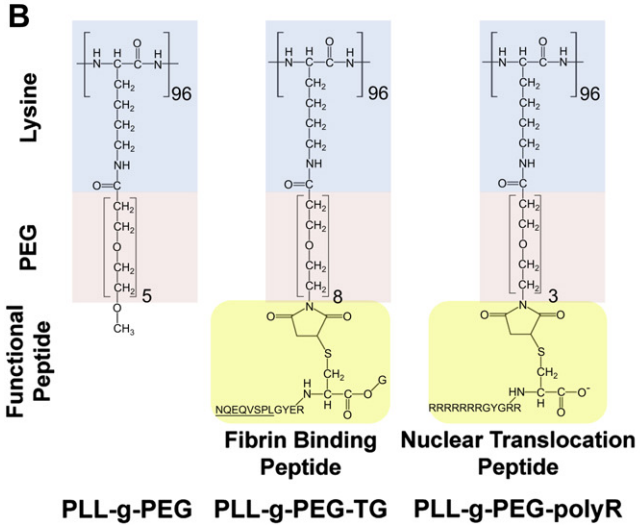

Fig. 1. Composition and structure of different PLL-g-PEG polymers. **A** PLL-g-PEG polymers consist of a 20 kDa PLL backbone, which is grafted by PEG with a ratio ranging between 3 and 8%. Surface-functionalized polymers carry peptides, namely TG and polyR, at the distal end of PEG. **B** Schematic view of PLL-g-PEG polymers with the lysine backbone (blue) consisting of 96 residues, the 5 kDa PEG residue (red) and both TG and polyR peptides (yellow) shows, that the peptides bind to PEG-maleimide via a the available sulfhydryl group of cysteine (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).