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# TGFβ functionalized starPEG-heparin hydrogels modulate human dermal fibroblast growth and differentiation

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ABSTRACT

Hydrogels are promising biomaterials that can adapt easily to complex tissue entities. Furthermore, chemical modifications enable these hydrogels to become an instructive biomaterial to a variety of cell types. Human dermal fibroblasts play a pivotal role during wound healing, especially for the synthesis of novel dermal tissue replacing the primary fibrin clot. Thus, the control of growth and differentiation of dermal fibroblasts is important to modulate wound healing. In here, we utilized a versatile starPEG-heparin hydrogel platform that can be independently adjusted with respect to mechanical and biochemical properties for cultivating human dermal fibroblasts. Cell-based remodeling of the artificial matrix was ensured by using matrix metalloprotease (MMP) cleavable crosslinker peptides. Attachment and proliferation of fibroblasts on starPEG-heparin hydrogels of differing stiffness, density of pro-adhesive RGD peptides and MMP cleavable peptide linkers were tested. Binding and release of human TGFβ$_1$ as well as biological effect of the pre-adsorbed growth factor on fibroblast gene expression and myofibroblast differentiation were investigated. Hydrogels containing RGD peptides supported fibroblast attachment, spreading, proliferation matrix deposition and remodeling compared to hydrogels without any modifications. Reversibly conjugated TGFβ$_1$ was demonstrated to be constantly released from starPEG-heparin hydrogels for several days and capable of inducing myofibroblast differentiation of fibroblasts as determined by induction of collagen type I, ED-A-Fibronectin expression and incorporation of alpha smooth muscle actin and palladin into F-actin stress fibers. Taken together, customized starPEG-heparin hydrogels could be of value to promote dermal wound healing by stimulating growth and differentiation of human dermal fibroblasts.

**Statement of Significance**

The increasing number of people of advanced age within the population results in an increasing demand for the treatment of non-healing wounds. Hydrogels are promising biomaterials for the temporary closure of large tissue defects: They can adapt to complex tissue geometry and can be engineered for specific tissue needs. We used a starPEG-heparin hydrogel platform that can be independently adjusted to mechanical and biochemical characteristics. We investigated how these hydrogels can support attachment, proliferation and differentiation of dermal fibroblasts. After introducing adhesive peptides these hydrogels support cell attachment and proliferation. Moreover, TGFβ – an essential growth and differentiation factor for fibroblasts – can be immobilized reversibly and functionally on these hydrogels. Thus, starPEG-heparin hydrogels could be developed to bioactive temporary wound dressings.



*Abbreviations:* 2D, two dimensional; 3D, three dimensional; αSMA, alpha smooth muscle actin; CL, MMP-cleavable linker; dFb, dermal fibroblast; ECM, extracellular matrix; Hep, heparin; MFb, myofibroblast; MMP, metalloprotease; PDGF, platelet derived growth factor; PEG, polyethylene glycol; RGD, Arginine–glycine–aspartic acid sequence; RPS26, 40S ribosomal protein S26; TGFβ, transforming growth factor β1; VEGF, vascular endothelial growth factor.

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## 1. Introduction

Dermal fibroblasts (dFb) and extracellular matrix (ECM) components released by them play crucial roles during wound healing [1]. When skin is injured, dFb and putative precursor cells from adjacent intact dermis are attracted to the fibrin rich wound bed where dFb proliferate and differentiate to myofibroblasts upon stimulation by local cytokines. Platelet derived growth factor (PDGF) and transforming growth factor beta 1 (TGFβ) from platelets and macrophages are functional mediators supporting proliferation and differentiation respectively [2,3]. Then, dFb differentiate to alpha smooth muscle actin (αSMA) expressing myofibroblasts (MFb) in the presence of TGFβ and mechanical stress [2,4]. Differentiated MFb are able to contract the wounds and to synthesize major components of the granulation tissue like collagen fibers and fibronectin [2,5].

In case of large wounds (e.g. burns) or disturbed wound healing (e.g. diabetes, infection or chronic venous insufficiency) engineered biomaterials may provide fast and sterile wound closure and initiate endogenous wound repair. In that context biomaterials should form a beneficial micro-milieu that provides key functions of the natural extra-cellular environment supporting adhesion, proliferation and differentiation of dFb. Here, hydrogels are of particular interest since they can mimic several physical properties of the skin and easily adapt to the size and shape of the wound due to their tuneable physical properties and high water content (more than 95% is water).

Thus, cell-instructive hydrogels enabling the independent control of physical cues, cell adhesion and the delivery of growth factors may be beneficial to control dermal fibroblast cell fate and therefore modulate wound healing (Fig. 1). Especially, the administration of TGFß from hydrogel matrices displaying a high cytokine binding and releasing capacity could be advantageous for myofibroblast activation, matrix deposition and wound closure. Within this study, a previously established modular hydrogel material based on star-shaped poly(ethylene glycol) (starPEG) and heparin [6] was investigated as an adaptable material to support growth and differentiation of human dermal fibroblasts. Both building blocks are widely applied in the biomaterials field because of their good compatibility [7,8]. The rational design concept of the starPEG-heparin hydrogels allows for the modulation of mechanical properties at constant heparin concentration [9], enabling cell responsiveness (via MMP-sensitive linkers [10,11]), adhesiveness (e.g. by incorporation of fibronectin derived adhesion peptides such as RGD) and cytokine presentation [12,13] independent of the network characteristics (stiffness, mesh size, hydration [9]). As a key feature, heparin allows the reversible conjugation of cytokines and mediators for stimulating cells involved in the healing process [14–16]. Especially the electrostatic interaction of specific amino acids of the TGFβ molecule with heparin enables the effective administration and protection of the protein as it might be beneficial for wound healing application [17–19].

In this study, we aimed to identify an optimal set of mechanical and biomolecular properties of starPEG-heparin hydrogels to support growth and differentiation of dFb for the promotion of wound healing. We tested different stiff hydrogels modified with RGD peptides, cross-linked with enzymatically cleavable peptides (CL) and with TGFβ conjugation to induce myofibroblast differentiation. The differently modified hydrogels were investigated concerning TGFβ binding and release, dFb spreading, attachment, proliferation, and differentiation after stimulation with hydrogel-adsorbed TGFβ.

## 2. Materials and methods

### 2.1. Cell culture experiments

The investigations were approved by the local ethics committee (065-2009) and conducted according to the Declaration of Helsinki Principles (1975). Primary human dFb from healthy breast skin or foreskin were isolated as previously described [20] by dispase II (Roche Diagnostics GmbH, Mannheim, Germany) mediated removal of the epidermal sheet and digestion of the dermal compartment with collagenase (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). To remove tissue debris the cell suspension was passed through 70 μM filters (BD Biosciences, Bedford, MA, USA). Cells were cultured with Dulbecco's Modified Eagle Medium (DMEM, Biochrom AG, Berlin, Germany) supplemented with 10% fetal calf serum (FCS) (Biochrom) and 1% penicillin/streptomycin (PAA Laboratories GmbH, Pasching, Austria) at 37 °C, 5% $CO_2$ until confluence. For experiments cells between passages 2–4 were used and if not mentioned otherwise dFb were detached by 0.05% trypsin/0.02% EDTA (Biochrom).

For growth on starPEG-heparin hydrogels, either synthetic medium (FibroLife Basal Medium® (Cell Systems, Troisdorf, Germany) supplemented with FibroLife Serum Free LifeFactor Kits® (containing 500 μg/ml of human serum albumin, 0.6 μM linoleic acid, 0.6 μg/ml lecithin, 5 ng/ml rhFGF, 5 ng/ml rhEGF, 30 pg/ml rhTGFβ, 50 μg/ml ascorbic acid, 7.5 mM L-glutamine and 1.0 μg/ml hydrocortisone hemisuccinate as final concentrations) or DMEM supplemented with 5% FCS and antibiotics was used as indicated. The day before seeding of the dFb on hydrogels, the cells were conditioned with synthetic medium.

#### 2.1.1. Preparation of StarPEG-heparin hydrogels

Three different types of hydrogels with or without RGD peptides and CL were prepared as planar layers of approximately 300 μm thickness. Synthesis of the of MMP cleavable peptide, starPEG-MMP conjugates, starPEG-heparin hydrogels and modification with RGD peptide were performed as previously described [9,21].

In brief, heparin (MW 14 000, Calbiochem (Merck, Darmstadt, Germany), EDC (Sigma–Aldrich, München, Germany), N-hydroxysulfosuccinimide (sulpho-NHS, Sigma–Aldrich) and amine end-functionalized 4-arm starPEG (MW 10 000, Polymer Source, Inc., Dorval, Canada) were dissolved in deionized, decarbonized water (MilliQ-water) on ice. After mixing the heparin with EDC and sulpho-NHS (2:1 ratio of EDC:sulpho-NHS), the solution was incubated on ice for 15 min. Following the activation of the heparin carboxylic acid groups, 4-arm starPEG or 4-arm starPEG-MMP conjugate was added and quickly vortexed. The liquid gel mixture was added either directly into a standard tissue culture plastic well plate or covalently attached onto amino-silanize glass coverslips. To ensure an even surface, a 0.025 mm height E-CTFE disc (Goodfellow GmbH, Bad Nauheim, Germany) was added onto of the polymerizing gel. After a polymerization time of 12 h, the E-CTFE films were removed and the hydrogels were washed and swollen in phosphate buffered saline (PBS) for 24 h. Hydrogels were adjusted by varying the molar ratio of starPEG to heparin ($\gamma$) and therefore the crosslinking degree to storage moduli of 1 ± 0.5 kPa ($\gamma$ = 1.5) or 10 ± 1 kPa ($\gamma$ = 3) (referring to a young's modulus of 3 or 30 kPa assuming a poisson ratio of 0.5 [22]) for cell experiments and to $\gamma$ = 2, $\gamma$ = 3 and $\gamma$ = 6 for TGFβ uptake and release experiments. Storage moduli of the hydrogels were determined as previously described [6]. The ratio of RGD peptides were adjusted to 1 mol RGD/mol heparin.

#### 2.1.2. Preparation of collagen-coated matrices for control

Collagen-coated tissue culture substrates were generated by *in vitro* fibrillogenesis of collagen from calf skin (Collagen A, Biochrom) according to manufacturer's instructions.

### 2.2. Attachment assay

$1.5 \times 10^4$ cells were seeded on each hydrogels-coated coverslips placed in 24-well format and incubated for 6, 24 and 48 h in