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TGF-β1-Modified Hyaluronic Acid/Poly(glycidol) Hydrogels for Chondrogenic Differentiation of Human Mesenchymal Stromal Cells

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In cartilage regeneration, the biomimetic functionalization of hydrogels with growth factors is a promising approach to improve the in vivo performance and furthermore the clinical potential of these materials. In order to achieve this without compromising network properties, multifunctional linear poly(glycidol) acrylate (PG-Acr) is synthesized and utilized as crosslinker for hydrogel formation with thiol-functionalized hyaluronic acid via Michael-type addition. As proof-of-principle for a bioactivation, transforming growth factor-beta 1 (TGF-β1) is covalently bound to PG-Acr via Traut's reagent which does not compromise the hydrogel gelation and swelling behavior. Human mesenchymal stromal cells (MSCs) embedded within these bioactive hydrogels show a distinct dose-dependent chondrogenesis. Covalent incorporation of TGF-β1 significantly enhances the chondrogenic differentiation of MSCs compared to hydrogels with supplemented noncovalently bound TGF-β1. The observed chondrogenic response is similar to standard cell culture with TGF-β1 addition with each medium change. In general, multifunctional PG-Acr offers the opportunity to introduce a range of biomimetic modifications (peptides, growth factors) into hydrogels and, thus, appears as an attractive potential material for various applications in regenerative medicine.

1. Introduction

Articular cartilage has important functions in the joint including protection of bone surfaces and insuring smooth and fluent joint movement in weight bearing areas;[1] however, the self-healing capactiy of cartilage is limited.[2] Thus, as a result of a severe trauma or as a consequence of high loads in the joint during lifetime potentially associated with degenerative processes, the cartilage tissue becomes damaged and loses its mechanical integrity leading to the development of pain and nonhealing cartilage defects. While more than 20 million people in the US suffer from osteoarthritis[3] and the World Health Organization (WHO) expects osteoarthritis to be the fourth most common cause leading to disability in 2020,[4] the clinically available treatment options are still not completely satisfying. Therefore, in order to develop alternative cell-based treatment options in trauma and degenerative disease, cartilage is a target for various tissue engineering approaches.[5]

Hydrogels consisting of hyaluronic acid (HA) have been shown to be promising for cartilage tissue engineering approaches, since HA is amenable to chemical functionalization,[6] and its presence enhances cartilage-specific extracellular matrix (ECM) synthesis.[7] Prestwich and co-workers have used thiol-functionalized hyaluronic acid (HA-SH) and linear[8] or four-armed[9] end-functionalized polyethylene glycol (PEG) acrylates for hydrogel formation. The polymer precursors were crosslinked in situ via pH-dependent Michael addition,[10] furnishing a thioether linkage. Linear poly(glycidol) (PG) as a biocompatible and water-soluble structural analog[11] of PEG exhibits a hydroxy methylene group at each repeating unit, which itself can undergo multiple functionalizations. In comparison to the widely used end-modified PEG, side chain-modified PG can provide a much higher crosslinking density and more versatile options for biomimetic functionalization, without negatively affecting hydrogel formation. However, despite the apparent potential advantages, PG has not yet been commonly applied for cartilage engineering until now.

Transforming growth factor-beta 1 (TGF-β1), a disulfide-linked protein homodimer of ≈25.6 kDa, controls, together with other growth factors, the chondrogenic differentiation of mesenchymal stromal cells (MSCs).[12] The addition of TGF-β1 as cell culture supplement facilitates MSC chondrogenesis reproducibly and is well acknowledged as a crucial factor for

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the sustainability of chondrogenic differentiation.[13] Studies in which TGF-β1 or its latent form has been covalently bound to hydrogels led to improved cell performance in comparison to exogenous supply of the growth factor.[14–16] However, so far, hydrogels with covalently bound TGF-β1 have not been directly compared to hydrogels with the same amount of noncovalently bound TGF-β1, and thus just mixed into the hydrogel. Covalent incorporation of TGF-β1 may omit the necessity for repeated administration in vivo, possibly enhancing the clinical potential of hydrogels for cartilage regeneration.

In this study, we evaluated multifunctional PG for crosslinking HA-based hydrogels and covalent binding of TGF-β1 and subsequently assessed chondrogenic differentiation of MSCs. TGF-β1 was chosen as promising model substance, due to its crucial role in in vitro chondrogenesis. A similar modification with TGF-β1 has been shown also in previous studies[15] to be suitable, due to the distinct chondrogenic effects, to evaluate whether the biofunctionalization was successful or not. Specifically, a multifunctional PG acrylate (PG-Acr) was synthesized and HA-SH-based hydrogels crosslinked with PG-Acr via Michael addition were established. The hydrogels were characterized with regard to rheological properties, swelling behavior, and degradation. Subsequently, varying amounts of TGF-β1 were covalently bound to PG-Acr and incorporated into the hydrogels; constructs with noncovalently bound growth factor or TGF-β1 exogenously supplemented to the medium served as control. MSC chondrogenesis was extensively evaluated by histology, immunohistochemistry (IHC), quantitative biochemical assays, and quantitative real-time polymerase chain reaction (qRT-PCR), with regard to qualitative and quantitative ECM deposition and gene expression.

2. Experimental Section

2.1. Chemicals and Materials

Chemicals and materials were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA), if not stated otherwise.

2.1.1. PG-Acr Synthesis

Acrylic anhydride (Polysciences, Warrington, PA, USA), anhydrous *N*,*N*-dimethylformamide (>99.8%), benzoylated dialysis tubing (molecular weight cut-off (MWCO) 2 kDa), calcium hydride (92%, abcr GmbH, Karlsruhe, Germany), Biotech cellulose ester dialysis membrane (MWCO 1 kDa, Spectrum Laboratories. Inc., Los Angeles, CA, USA), ethanol (>99.8%), ethyl vinyl ether (99%, potassium hydroxide stabilized), glycidol (96%), potassium-*tert*-butoxide (KOtBu, 1 M in tetrahydrofurane), pyridine (>99.8%, anhydrous), *p*-toluene sulfonic acid monohydrate (pTsOH, >98.5%), sodium hydroxide, magnesium sulfate (p.a., Merck, Darmstadt, Germany), and hydrochloric acid (39%, Merck) were used.

2.1.2. Hydrogel Formulation

2-Iminothiolane (Traut's reagent), recombinant human TGF-β1 (>98%, BioLegend), HA-SH (Glycosil, ESI BIO, Alameda, CA, USA), silicone grease (medium viscous, Bayer, Leverkusen, Germany), glass cylinders (inner diameter 6 mm, length 8 mm, Hilgenberg) were used. Ultrapure water (H_2O, resistance >18.2 MΩ cm) was purified with Sartorius Arium Pro (Sartorius AG, Goettingen, Germany).

2.1.3. Cell Culture

L-Ascorbic acid 2-phosphate sequimagnesium salt hydrate, basic fibroblast growth factor (bFGF; BioLegend, London, UK), dexamethasone, Dulbecco's Modified Eagle's Medium (DMEM) high glucose 4.5 g L^{-1}, Dulbecco's Modified Eagle's Medium/Ham's F-12 (DMEM/F12) (Thermo Scientific, Waltham, USA), fetal bovine serum (FBS) (Thermo Scientific, Waltham, USA), ITS+ Premix (Corning; NY, USA), Live/Dead cell staining kit (PromoKine, Heidelberg, Germany), penicillin–streptomycin (PS; 100 U mL^{-1} penicillin, 0.1 mg mL^{-1} streptomycin) (Thermo Scientific, Waltham, USA), phosphate-buffered saline (PBS; Life Technologies, Karlsruhe, Germany), L-proline, sodium pyruvate, and 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) (Thermo Scientific, Waltham, USA) were used as received.

As primary antibodies, anti-aggrecan 969D4D11 (Thermo Scientific, Waltham, USA), anti-collagen I ab34710 (Abcam, Cambridge, UK), anti-collagen II II-4C11 (Acris, Herford, Germany), and anti-collagen X X53 (Thermo Scientific, Waltham, USA) were used.

As secondary antibodies, donkey anti-mouse (Cy3; Dianova, Hamburg, Germany), and goat anti-rabbit (Alexa Fluor 488; Dianova, Hamburg, Germany) were used. Furthermore, antibody diluent Dako REAL (Dako, Hamburg, Germany), and 4′,6-diamidino-2-phenylindole (DAPI) mounting medium ImmunoSelect (Dako, Hamburg, Germany) were used.

Other reagents used in biological experiments: Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent, Santa Clara, USA), bovine chondroitin sulfate (Sigma-Aldrich, St. Louis, MO, USA), chloramine T (Carl Roth, Karlsruhe, Germany), *p*-dimethylamino-benzaldehyde (DAB) (Carl Roth, Karlsruhe, Germany), dimethylmethylene blue (DMMB), fast green, Hoechst 33258, L-hydroxyproline, ImProm-II Reverse Transcription System (Promega, Madison, USA), papain (Worthington, Lakewood, USA), picric acid solution, Proteinase K (Digest-All 4, Life Technologies Karlsruhe, Germany), Tissue Tek Optimal Cutting Temperature compound (O.C.T., Sakura Finetek, Tokyo, Japan), TRIzol Reagent (Thermo Scientific, Waltham, USA), and safranin O were used as received.

2.2. Analytical Instruments and Preparation

^{1}H NMR spectra were recorded on a Bruker Fourier 300. Deuterated chloroform ($CDCl_3$) and deuterated water (D_2O) spectra were recorded with nondeuterated solvent signals of $CDCl_3$ (7.24 ppm) and 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (0.00 ppm) as an internal reference, respectively.

Size exclusion chromatography (SEC) elugrams in dimethylformamid (DMF) (containing 1 g L^{-1} LiBr) were recorded with an agilent 1260 infinity multi-detector suite from Polymer Standards Service system (PSS, Mainz, Germany) with a

50 mm PFG precolumn and three 300 mm PFG columns (pore size 7 µm) and a column oven at 40 °C. The elution rate was 1 mL min^{-1} and calibration was performed using poly(ethylene glycol) standards (PSS, Mainz, Germany). Data was processed with WinGPC software. Samples of the raw polymers and the mercaptoethanol quenched acrylate derivatives were previously filtered through 0.2 µm polytetrafluoroethylene (PTFE) syringe filters (Roth, Karlsruhe, Germany). Rheological properties of the hydrogels were evaluated using a Physica MCR 301 rheometer from Anton Paar (Graz, Austria) equipped with a parallel-plate geometry. The diameter of the plate was 25 mm and the plate to plate distance was 0.5 mm. Freeze-drying of the polymers after dialysis was performed with an freeze dryer (Alpha 1–2 LD, Christ, Osterode am Harz, Germany).

2.3. Poly(glycidol) Synthesis and Characterization

The monomer ethoxyethyl glycidyl ether (EEGE) was synthesized according to an established procedure.[17] Glycidol and ethyl vinyl ether were cooled to 0 °C. pTsOH was slowly added, taking into account that the reaction temperature did not exceed 20 °C. The obtained reaction mixture was subsequently stirred for 3 h at RT. Afterward, the organic phase was washed with 3 × 50 mL saturated $NaHCO_3$ solution and dried with $MgSO_4$. Excess ethyl vinyl ether was removed using vacuum. The resulting product was further dried with CaH_2 and distilled at about 1 mbar, and 60 °C. EEGE was obtained as a colorless liquid and stored at 4 °C under argon atmosphere.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.295 (t, 3H, CH_3CH_2, ^3J = 7.0 Hz), 1.414 (m, 3H, CH_3CH), 2.688–2.748 (m, 1H, OCH_2CHCH_2), 2.894 (m, 1H, OCH_2CHCH_2), 3.188–3.292 (m, 1H, OCH), 3.480–3.932 (m, 4H, CH_3CH_2 and $OCHCH_2O$), 4.821–4.884 (m, 1H, $CHCH_3$) (Figure S1, Supporting Information)

The linear poly(ethoxyethyl glycidyl ether) (PEEGE) was synthesized in bulk using 50 equivalents of EEGE (10 mL, 9.62 g, 65.806 mmol) on one equivalent KOtBu initiator (1.316 mmol, 1.3158 mL 1 M KOtBu in tetrahydrofurane (THF)). For the reaction, KOtBu was placed under argon atmosphere, and EEGE was added afterward, the obtained mixture was subsequently stirred for 1 d at 60 °C. The polymerization reaction was stopped by addition of EtOH.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.119 (t, 3H, CH_3CH_2, ^3J = 6.95 Hz), 1.2165 (m, 3H, CH_3CH), 3.320–3.658 (m, 7H, CH_2CH, CH_2CH, CH_2CH, CH_3CH_2), 4.582–4.671 (m, 1H, CH_3CH) (Figure S2, Supporting Information)

For deprotection, the linear polymer PEEGE was dissolved in a minimal volume of ethanol and concentrated hydrochloric acid (37%) was added and stirred overnight at room temperature. The solution was neutralized with 1.0 N NaOH, dialyzed against H_2O (MWCO 1 kDa) for 2 d (10 × 2 L), and subsequently freeze-dried. The yield of the resulting PG over two reaction steps (polymerization and deprotection) was about 70%, depending on the applied dialysis time.

^{1}H NMR (300 MHz, D_2O, δ): 1.221 (s, 9H, ^{t}Bu), 3.577–3.784 (m, 5H)

For the preparation of functional polymers, the deprotected linear PG was dissolved in dry DMF. 0.2 equivalents of pyridine and acrylic acid were added (on free hydroxyl groups of PG) and stirred overnight in the dark. For neutralization 1 mL of a 0.8 M phosphate buffer (pH 8.0) was added and stirred for 0.5 h. The final solution was dialyzed against ethanol (benzoylated dialysis tubing, MWCO 2 kDa) for 2 d (10 × 100 mL) with light exclusion. The ethanolic solution of PG-Acr was stored at 4 °C until further use. Right before application, the solvent was removed by rotary evaporation at room temperature after adding 10 mg phenothiazine as a stabilizer to about 5 mL of the PG-Acr solution.

^{1}H NMR (300 MHz, D_2O, δ) 1.238 (s, ^{t}Bu), 3.330–4.041 (m, 5H, PG, CH_2OH), 4.235–4.564 (m, 2H, $COOCH_2$), 6.024–6.059 (m, 1H, $OCOCHCH_2$, *cis*), 6.209–6.301 (m, 1H, $COCHCH_2$), 6.455–6.513 (m, 1H, $OCOCHCH_2$, *trans*)

2.4. Hydrogel Formulation and Characterization

2.4.1. Hydrogel Preparation Procedure and Growth Factor Incorporation

Before hydrogel formulation, HA-SH (Glycosil) stock solution (10 mg mL^{-1} in PBS, according to the manufacturer's instructions) and PG-Acr stock solution (125 mg mL^{-1} in PBS, sparingly soluble phenothiazine was afterward removed by centrifugation) were prepared. Three different kinds of hydrogels were prepared: gels without TGF-β1, gels supplemented with 100 nM noncovalently bound TGF-β1, and gels that were covalently modified with final concentrations of 10 nM, 50 nM, and 100 nM tethered TGF-β1.

In the case of the nonloaded hydrogels, PBS was added to the PG-Acr stock solution to adjust its concentration to 29.5 mg mL^{-1}. The hydrogels (n = 3 for each measurement) were formulated by mixing the HA-SH stock solution with the PG-Acr solution in a volume ratio of 4:1.

For covalent binding of TGF-β1 to the hydrogels, first, TGF-β1 was thiol-modified using Traut's reagent at a molar ratio of 4:1 of Traut's reagent to TGF-β1 for 1 h at RT. Subsequently, various doses of thiolated TGF-β1 were coupled to PG-Acr for 1 h at 37 °C, obtaining TGF-β1 containing PG-Acr solutions (29.5 mg mL^{-1} in PBS). Finally, the hydrogels were formulated by mixing the HA-SH stock solution with the differently TGF-β1-modified PG-Acr solutions resulting in final concentrations of 10 nM, 50 nM, and 100 nM tethered TGF-β1 (10 nM, 50 nM, and 100 nM TGF-β1 + Traut).

For the preparation of hydrogels containing 100 nM of noncovalently bound TGF-β1 (100 nM TGF-β1) a TGF-β1 containing PG-Acr solution (29.5 mg mL^{-1} in PBS) was prepared as described above, but without thiol-modification of TGF-β1 with Traut's reagent.

2.4.2. Rheological Characterization

Oscillatory measurements with a standard plate-plate geometry of 25 mm diameter were performed at 20 °C with 400 µL of the hydrogel precursor solution. Time sweeps (angular frequency = 1 rad s^{-1}, strain = 1%) were performed over a time period of 90 min. The gelation point was determined at the intersect of the elastic and viscous modulus. Amplitude

(angular frequency = 10 rad s^{-1}, strain 0.1–10%) and frequency (angular frequency = 100–0.1 rad s^{-1}, strain = 1%) sweeps were performed immediately after the time sweeps to verify the correct measurements of the hydrogels. A water-soaked paper towel and a cap were used to prevent drying-out of the hydrogel solution during the measurements.

2.4.3. Hydrogel Swelling, Sol Fraction, and Degradation Measurements

For in vitro swelling measurements, hydrogels with 100 nM covalently bound TGF-β1 and w/o TGF-β1 with a final volume of 150 µL were mixed and the precursor solutions were filled into glass cylinders with a diameter of 6 mm and a height of 8 mm, which were sealed to the bottom with silicone grease and afterward incubated at 37 °C for 0.5 h. After 0.5 h the premature hydrogels were removed from the glass cylinders, weighed and put in microcentrifuge cups with 2 mL PBS and incubated at 37 °C without shaking. Triplets of each hydrogel type were produced for each time point (0.25 d and 1, 4, 7, 14, and 21 d). To determine the swelling ratio and sol fraction, hydrogels and solvent were separated and excessive PBS was removed carefully from the hydrogel surface using a filter paper and the swollen weight (W_s) was evaluated. Subsequently, the hydrogels were freeze-dried, the weight of the dry hydrogel (W_d) was determined, and the swelling ratio (Q_m) was calculated according to Equation (1).

$$Q_m = \frac{W_s}{W_d} \tag{1}$$

2.4.4. Quantification of TGF-β1 Release

To compare the amount of total released TGF-β1 from hydrogels loaded with 100 nM of tethered TGF-β1 (100 nM TGF-β1 + Traut) and 100 nM of noncovalently bound TGF-β1 (100 nM TGF-β1), cell-free hydrogels were prepared as described above (Section 2.4.1). The respective hydrogels (V = 40 µL) were maintained in chondrogenic medium, w/o soluble TGF-β1, over a time course of 21 d. The supernatants of the hydrogels were collected at different time points (0.25, 1, 2, 4, 9, 11, 14, 16, 18, and 21 d) and stored at –20 °C until analysis. The amount of released TGF-β1 was measured using a LEGEND MAX Total TGF-β1 enzyme-linked immunosorbent assay (ELISA) Kit (BioLegend, London, UK) according to the manufacturer instructions.

2.4.5. MSC Isolation and Expansion

Human bone marrow-derived MSCs were isolated from the surgically removed cancellous bone of patients undergoing total hip replacement, with written informed consent from all patients, and as approved by the local ethics committee. Briefly, MSCs were collected by repeated washing of bone debris and marrow in PBS, then centrifuged at 300 g for 10 min, resuspended in proliferation medium (DMEM/F12, supplemented with 10% FBS, 1% PS and 5 ng mL^{-1} bFGF), and seeded into T175 cm^2 flasks (Greiner Bio-One, Frickenhausen, Germany). After 2 d the cells were washed with PBS to remove nonadherent cells, and the adherent cells were subsequently cultured to a subconfluent level at 37 °C, 5% CO_2 in proliferation medium. For MSC passaging, cells were detached with 0.25% trypsin-EDTA and seeded at a density of ≈5000 cells mL^{-1} into T175 cm^2 flasks.

2.4.6. MSC Hydrogel Encapsulation

The hydrogel precursor solutions were prepared as described above (Section 2.4.1). Additionally, the PG-Acr stock solution (29.5 mg mL^{-1} in PBS) was sterile-filtered through a 0.2 µm syringe-filter prior utilization.

The MSCs were propagated up to passage 2 or 3 for hydrogel encapsulation experiments. Prior to crosslinking, MSCs were resuspended in the HA-SH solution, and either the differently TGF-β1-modified PG-Acr solutions (TGF-β1 + Traut), a PG-Acr solution containing 100 nM TGF-β1 w/o Traut's reagent (100 nM TGF-β1), or unmodified PG-Acr were added to the cell-laden HA-SH solutions at a final concentration of 20.0×10^6 MSC mL^{-1}. Finally, 40 µL of the HA-SH hydrogel solutions were filled into a glass ring (Ø 5 mm) and allowed to gel for 30 min at 37 °C, 5% CO_2. Afterward, hydrogel encapsulated MSCs were cultured for up to 21 d in chondrogenic medium (DMEM high glucose 4.5 g L^{-1}, supplemented with 1% ITS+ Premix, 40 µg mL^{-1} L-proline, 50 µg mL^{-1} L-ascorbic acid 2-phosphate sequimagnesium salt hydrate, 0.1×10^{-6} M dexamethasone, 1×10^{-3} M sodium pyruvate, and 1% PS), w/o soluble TGF-β1. Growth factor-unmodified PG-Acr hydrogels served as controls and were cultured in chondrogenic medium either supplemented with TGF-β1 (addition with each medium change, 10 ng mL^{-1}), as standard control for in vitro chondrogenesis of MSC (TGF-β1 Medium), or in chondrogenic medium w/o TGF-β1 (w/o TGF-β1), as a negative control.

2.4.7. Cell Viability Assay

The viability of encapsulated MSCs was assessed using a Live/Dead cell staining kit. At day 2, 10, and 21 after encapsulation, cell-laden hydrogels were washed with PBS and incubated in the Live/Dead staining solution (4×10^{-6} M ethidium homodimer III (EthD-III), 2×10^{-6} M calcein acetoxymethyl ester (Calcein-AM)) for 45 min. Following that, top view images were captured immediately using a fluorescence microscope (Olympus BX51/DP71, Olympus, Hamburg, Germany).

2.4.8. Histological and Immunohistochemical Analyses

The hydrogels were initially fixed in 3.7% PBS-buffered formalin for 60 min, washed two times in PBS for 15 min, and incubated in O.C.T. overnight at 4 °C. On the next day, constructs were frozen in cryomolds containing fresh O.C.T. using liquid nitrogen and stored at –80 °C until sectioned.[18] Longitudinal sections at 8 µm were prepared and collected on Super Frost plus glass slides (R. Langenbrinck, Emmendingen,

Germany). For histological evaluation the samples were stained with either Weigert's hematoxylin, fast green, and safranin O for deposition of glycosaminoglycans (GAG)[19] or Weigert's hematoxylin and picrosirius red for collagen deposition.[20]

For immunohistochemical analyses, the cryosections were rehydrated, and antigen retrieval was performed using Proteinase K for 10 min at RT. Subsequently, sections were blocked with 1% bovine serum albumin (BSA) for 30 min; primary antibodies were diluted in Antibody diluent Dako REAL and incubated overnight in a humidified chamber at RT. Antibodies for collagen type I (ab34710, 1:800), collagen type II (II-4C11, 1:100), collagen type X (X53, 1:200), and aggrecan (969D4D11, 1:300) were used. Sections were washed three times in PBS for 3 min, and secondary antibodies were diluted in Antibody diluent Dako REAL and applied in the dark for 1 h. A donkey anti-mouse (Cy3, 1:500) and a goat anti-rabbit (Alexa Fluor 488, 1:400) secondary antibody were used. Subsequently, the slides were washed three times in PBS for 3 min and mounted with DAPI mounting medium ImmunoSelect. Images were captured with a fluorescence microscope (Olympus BX51, Olympus, Hamburg, Germany).

2.4.9. Biochemical Analyses

For biochemical analyses, the constructs were digested in 1 mL of a papain solution (3 U mL^{-1}) for 16 h at 60 °C. Prior to papain digestion the gels were homogenized at 25 Hz for 5 min using a TissueLyser (Qiagen, Hilden, Germany).

DNA content of digested constructs was measured using Hoechst 33258 DNA intercalating dye. DNA quantification was carried out fluorometrically at 340 and 465 nm, using salmon testis as standard.[21] The amount of GAG produced by the encapsulated cells was measured using the DMMB assay. The GAG amount was determined spectrophotometrically at 525 nm, using bovine chondroitin sulfate as standard.[22] The content of hydroxyproline was measured, after acid hydrolysis and reaction with DAB and chloramine T. The quantification was adapted to 96-well format and carried out with a spectrophotometer at 570 nm, using L-hydroxyproline as standard. The amount of collagen was calculated using a hydroxyproline to collagen ratio of 1:10.[23,24]

2.4.10. Gene Expression

Before RNA isolation, cell-laden hydrogels, and cells of 2D samples at day 0 were homogenized in TRIzol Reagent, and RNA was subsequently isolated according to the manufacturer's instructions. First-strand cDNA was synthesized from total RNA by using the ImProm-II Reverse Transcription System. Brilliant III Ultra-Fast SYBR Green QPCR Master Mix was used for qRT-PCR analysis. qRT-PCR was carried out using PrimePCR SYBR Green Assay (Bio-Rad, München, Germany) primer pairs for aggrecan (*ACAN*, qHsaCID0008122), collagen I (*COL1A1*, qHsaCED0043248), collagen II (*COL2A1*, qHsaCED0001057), collagen X (*COL10A1*, qHsaCED0043992), Sox9 (*SOX9*, qHsaCED0021217), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, qHsaCED0038674), according to the manufacturer's cycle instructions. Expression of all genes was normalized to the housekeeping gene *GAPDH* for each construct group and time point. The *x*-fold increase in gene expression levels for each gene was determined using the $2^{-\Delta\Delta CT}$ method and further normalized to cells from 2D samples at day 0.

2.4.11. Statistics

Statistics was performed using GraphPad Prism, Version 6.0 (GraphPad Software, La Jolla, USA). Results are presented as mean values ± standard deviation (SD). Statistical significance was assessed by either multiple *t*-tests followed by Holm–Sidak post hoc test, or by two-way analysis of variance followed by Tukey's post hoc test, as appropriate. The statistical significance level was set at $p < 0.05$.

3. Results and Discussion

3.1. Poly(glycidol) Synthesis and Characterization

Linear homopolymeric PG with 1-ethoxyethyl protection of the alcohol groups was obtained by anionic ring-opening polymerization of ethyl glycidyl ether with potassium *tert*-butanoate as initiator via solventless synthesis with a monomer to initiator ratio of 50:1. ^{1}H-NMR analysis confirmed complete monomer conversion and the structure of the obtained polymer (Figure S2, Supporting Information). After polymerization, the ethoxyethyl protecting groups were completely removed by treatment with hydrochloric acid. The PG alcohol groups were afterward partially converted to PG-Acr by reaction with 0.2 equivalents of acrylic anhydride catalyzed by pyridine (**Figure 1**).

To facilitate the work-up procedure, the resulting PG-Acr was afterward treated with 0.8 M phosphate buffer (pH 8.0) to decompose the formed organic pyridinium acrylate salt (pK_a = 5.25 in H_2O),[25] which otherwise might have a high binding affinity to the polyether structure of PG. Subsequently, the obtained solution was dialyzed against EtOH to minimize possible ester hydrolysis. The obtained ethanolic solution was used to store the polymer in the dark for longer periods of time. Before application, the solvent had to be removed, which was performed by addition of phenothiazine to prevent an early

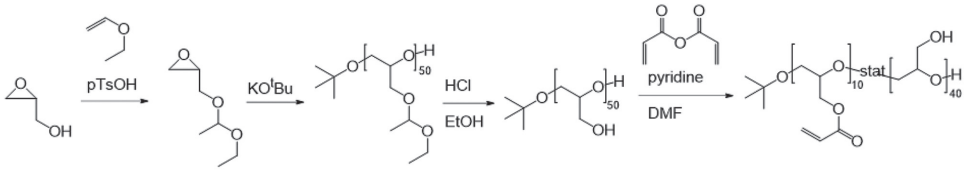

Figure 1. Synthetic route to PG-Acr.

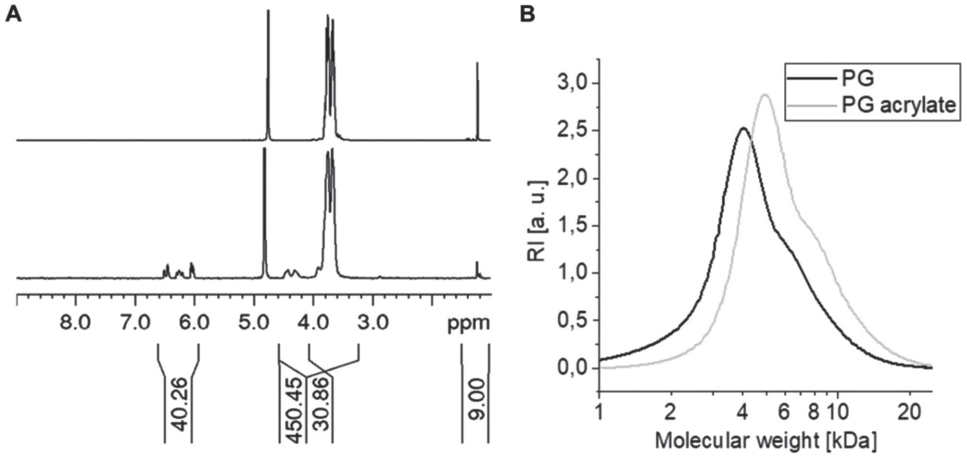

Figure 2. Characterization of the multifunctional PG-Acr crosslinkers. A) ^{1}H NMR spectra (D_2O) of PG (top) and PG-Acr (bottom) to show acrylate modification. B) Molecular weight distributions determined by SEC measurements (DMF) of PG and PG-Acr to show the molecular weight increase after successful acrylation.

radical crosslinking of the reactive acrylate groups during the procedure. Phenothiazine (soluble in water with ≈1.7 mg L^{-1})[26] precipitated after the dissolution of the mixture in aqueous solvent and could easily be removed by centrifugation. ^{1}H NMR and SEC analysis (**Figure 2**) demonstrated that, as desired, the obtained degree of functionalization was 20% ± 2% (estimated by comparison of the ^{1}H NMR integrals of the CH_2 group next to the ester and the PG backbone groups), providing a sufficient amount of acrylate functions for crosslinking and biomimetic functionalization. In comparison to the used PG homopolymer ($Đ$ = 1.31), the molecular weight distribution of the PG-Acr ($Đ$ = 1.18) did not show enhanced dispersity, indicating the effective protection of the acrylates by the added stabilizer.

3.2. Hydrogel Formation and Characterization

Hydrogels were formed by crosslinking of the linear polymeric precursors PG-Acr and HA-SH solution via the pH- and buffer-dependent Michael addition crosslinking reaction.[10,27] A possible side reaction is oxidative linking of thiols to disulfide bridges. Due to the large differences in reaction kinetics in favor of Michael addition, the contribution of disulfide formation between modified hyaluronic acid molecules is negligible for the network formation. In order to prepare biomimetic hydrogels, nucleophilic groups (for example free lysine amine groups) of TGF-β1 were first reacted with Traut's reagent (**Figure 3**) in a 4:1 molar ratio of Traut's reagent to TGF-β1, as described in literature,[15] followed by reacting the resulting conjugate with PG-Acr. The resulting PG-Acr-TGF-β1 conjugates were then used for hydrogel formation with the HA-SH solution (and cells in cases of cell experiments) to yield an overall concentration of 10 nM, 50 nM, or 100 nM TGF-β1 in the hydrogel. The resulting overall polymer content of the hydrogels was 1.388% (w/v), with 0.8% (w/v) for HA-SH and 0.588% (w/v) for PG-Acr, representing a low mass percentage hydrogel, likely providing a good penetrability for cellularly produced ECM.[28,29]

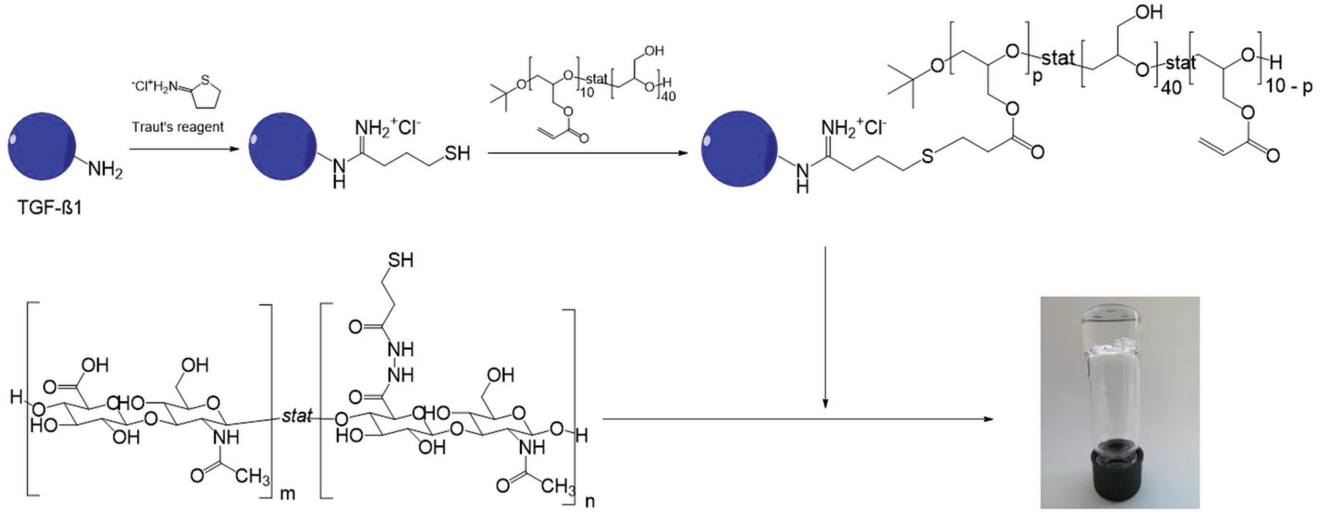

Figure 3. In situ functionalization of PG-Acr with TGF-β1 via Traut's reagent and crosslinking with the thiol-functionalized hyaluronic acid to bioactive hydrogels.

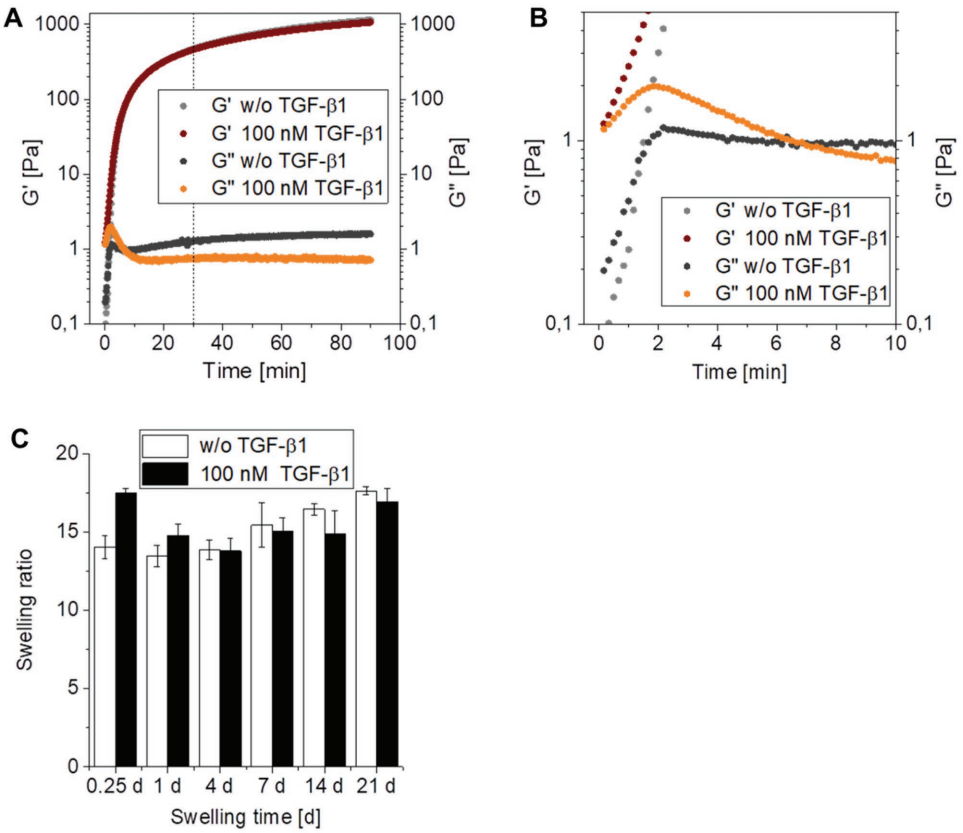

Figure 4. Characterization of the obtained hydrogels with covalently bound (100 nM TGF-β1) and without TGF-β1 (w/o TGF-β1). A) Representative rheological time sweep indicating the similar hydrogel stiffness with and without growth factor (dashed line indicate the time of prematuration in the glass cylinders). B) Magnification of the initial crosslinking phase indicating an early gel formation for both gels. C) Swelling ratio Q_m based on hydrogel mass.

By oscillatory rheometry (**Figure 4**A,B and Figure S3, Supporting Information), information about the crosslinking kinetics and fluid-solid properties during the hydrogel formation were gained. Hydrogels with and without 100 nM TGF-β1 were formulated by mixing the viscous HA-SH stock solution with the low viscous PG-Acr or the PG-Acr/TGF-β1 stock solution. The resulting rheograms showed no significant differences between hydrogels with and without covalently bound 100 nM TGF-β1, both for the kinetics of gelation and the finally achieved elastic moduli. The elastic modulus in both cases was about 500 Pa after 30 min and 1100 Pa after 90 min of gelation. These results demonstrate the suitability of side-chain modified PG for covalent binding of biomimetic cues such as growth factors without compromising the gelation process. Even at the highest investigated concentration of TGF-β1, the rheological properties of the resulting hydrogels were not affected. Furthermore, the gelation profile showed that the hydrogel system provided a suitable time window between the gelation point at about 2 min, after which embedded cells do not rapidly sink to the bottom any more, and an ongoing gelation and crosslinking for over 1 h, to result in well-distributed cells incorporated within the hydrogels. The hydrogels were observed to be considerably softer in comparison to native cartilage, for which an elastic modulus of ≈0.4–0.6 MPa was reported.[30] However, a hydrogel stiffness similar to that determined for the hydrogels developed in this study has previously been shown to be suitable in cartilage engineering for robust chondrogenic differentiation of MSCs.[31,32]

For further hydrogel characterization, the swelling ratios of the hydrogels, with and without covalently incorporated 100 nM TGF-β1, were evaluated at different incubation time points (0.25, 1, 4, 7, 14, and 21 d). As shown in Figure 4C, in general there was no significant difference between hydrogels without (0.25 d: 14.0 ± 0.7 and 21 d: 17.6 ± 0.3) and those with 100 nM TGF-β1 (0.25 d: 17.5 ± 0.3 and 21 d: 16.9 ± 0.8) at early and late time points. No hydrogel dissolved within the time frame of 21 d, and swelling ratio only slightly increased over time, which indicated a progressive loss of hydrogel integrity, possibly due to ester hydrolysis and subsequent water gain. As ester bonds close to the thioester are known to be prone to hydrolysis,[33] hydrolysis experiments with PG-Acr and PG mercaptoethanol as a model compound for the converted PG acrylate groups were performed. After three weeks, around 90% of the ester groups were still maintained. The weight of the released sol fraction was evaluated additionally and showed no significant increase within three weeks (data not shown), which one could expect for a multianchored polymeric network, emphasizing the benefits of employing multiple side chain-functionalized PG-Acr in comparison to the commonly used bifunctional PEG diacrylates or the tetrafunctional starPEG acrylates.[11,34]

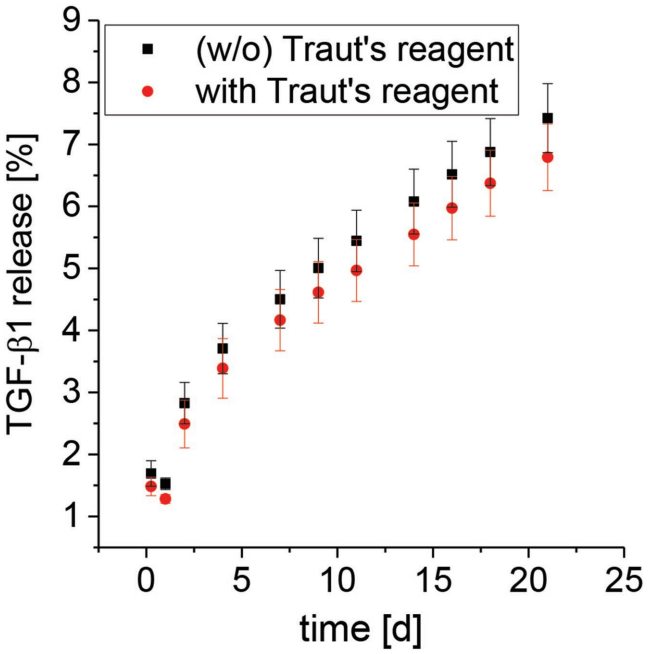

Figure 5. Cumulative release of 100 nM covalently bound and noncovalently bound TGF-β1 from the hydrogels, determined by ELISA.

To further characterize the hydrogel systems, the amount of TGF-β1 released from cell-free hydrogels was determined via ELISA. Hydrogels loaded with covalently bound TGF-β1 (100 nM TGF-β1 + Traut) were compared to hydrogels loaded with the same amount of noncovalently bound TGF-β1 (100 nM TGF-β1). An ELISA of the initially incorporated TGF-β1 solution yielded correct TGF-β1 concentrations during growth factor loading into the hydrogels (data not shown). The supernatants of the respective hydrogels were collected over a time course of 21 d. The resulting cumulative release curves showed no significant differences between hydrogels with covalently bound and noncovalently bound TGF-β1 (**Figure 5**). In both cases no initial burst release occurred. Only a 7–8% overall release of TGF-β1 was detectable after 21 d. Possible reasons for the relatively low release, especially of the noncovalently bound protein, may include electrostatic interactions of TGF-β1 with the hyaluronic acid component of the gels, or an effective diffusion inhibition by the hydrogel network.

3.3. Chondrogenic Differentiation of MSCs within the HA/PG Hydrogels

After the successful establishment and characterization of the PG-Acr crosslinked HA-SH-based hydrogel system, we evaluated the chondrogenic response of human MSCs to biomimetic hydrogels incorporating TGF-β1 as model substance. Varying concentrations of TGF-β1 were covalently bound to the hydrogels (TGF-β1 + Traut group). Additionally, the effect on MSC chondrogenesis was compared to hydrogels with noncovalently bound TGF-β1 (TGF-β1 group) and, as a standard control, TGF-β1 supplemented with each medium change (10 ng mL^{-1}; TGF-β1 Medium group).

All groups exhibited high amounts of viable cells during the 21 d of in vitro chondrogenesis, and we did not detect significant differences between the different hydrogel groups regarding cell viability (Figure S4, Supporting Information).

3.3.1. Determination of GAG Production

The determination of GAG production, either quantitatively using DMMB assay or histologically using safranin O, showed that hydrogels cultured w/o TGF-β1 produced, as expected, no significant amounts of GAG (**Figure 6**). 10 nM and 50 nM TGF-β1 + Traut-modified gels showed no, or very little GAG deposition in the safranin O staining (Figure S5, Supporting Information), and no significant GAG production in the DMMB assay (Figure 6B). In contrast to that, the 100 nM TGF-β1 + Traut group showed an intense GAG deposition in histological images, which was distinctly stronger than that determined for the group with noncovalently bound TGF-β1 (100 nM TGF-β1) (Figure 6A). While the safranin O staining exhibited very strong pericellular signals in the 100 nM TGF-β1 + Traut group, GAGs seemed to be more evenly distributed throughout the gel in the TGF-β1 Medium group, but less intense (Figure 6A). Reflecting the histological results, quantification of GAG demonstrated that the 100 nM TGF-β1 + Traut group produced significantly higher amounts of GAG/DNA (26.6 ± 2.3 µg/µg), as compared to the 100 nM TGF-β1 group (13.0 ± 2.7 µg/µg), indicating a beneficial effect of covalent incorporation of TGF-β1 on chondrogenesis compared to just mixing TGF-β1 into the gels (Figure 6B). In this regard, comparisons to previous studies employing covalently bound TGF-β1 are difficult, as a group with incorporated, but not covalently bound TGF-β1 was not investigated in these studies.[14–16] In the present study, interestingly, the group with 100 nM tethered TGF-β1 was superior to the TGF-β1 Medium control group (17.2 ± 3.1 µg/µg) after 21 d of chondrogenic differentiation (Figure 6B). This result was in agreement with observations reported for purely PEG-based hydrogel systems with tethered TGF-β1 compared to TGF-β1-supplemented medium, using either MSC or chondrocytes.[14,15] In this study, in order to covalently bind TGF-β1, the growth factor was thiol-modified using Traut's reagent at a molar ratio of 4:1 of Traut's reagent to TGF-β1 which, as demonstrated previously, does not impair TGF-β1 bioactivity and functionality when compared to native TGF-β1.[14,15]

3.3.2. Determination of Collagen Production

MSCs cultured in gels w/o TGF-β1 synthesized very little amounts of collagens, as shown by hydroxyproline assay and picrosirius red staining (**Figure 7**). Moreover, collagen staining for the 10 nM and 50 nM TGF-β1 + Traut-modified groups resulted in very weak signals (Figure S6, Supporting Information), and substantially lower production of collagens compared to the other chondrogenically induced groups (Figure 7B). Strong picrosirius red staining was detected for the 100 nM TGF-β1 + Traut group. Similar staining, but slightly more homogeneously distributed throughout the gel matrix, was observed for the TGF-β1

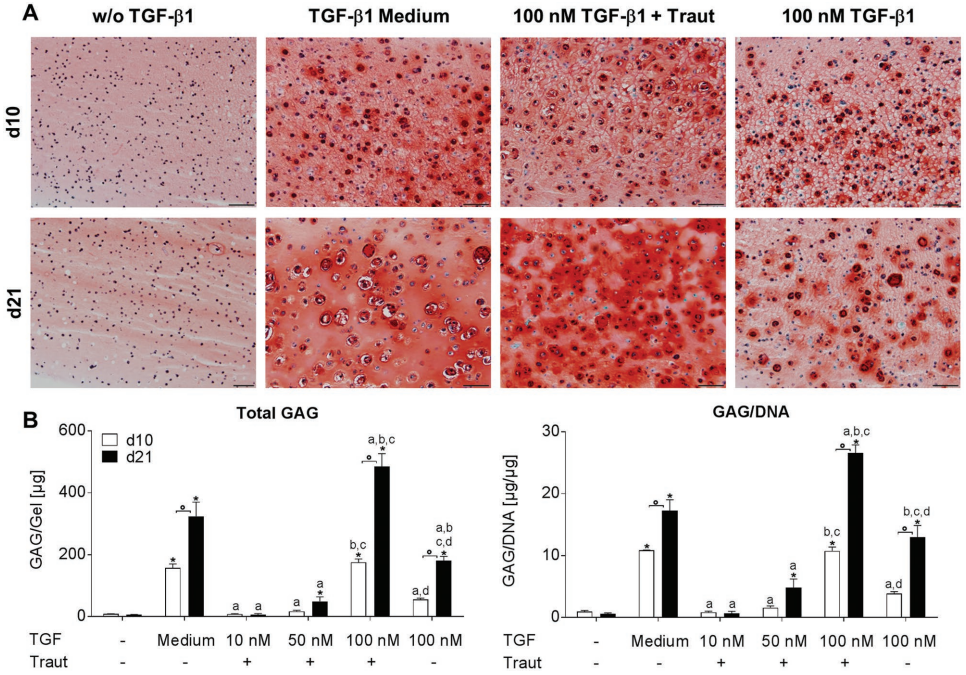

Figure 6. A) Histological stainings of TGF-β1-laden hydrogels, seeded with 20.0×10^6 MSCs mL^{-1}, after 10 and 21 d of chondrogenic differentiation. Longitudinal sections were stained for deposition of glycosaminoglycans with safranin O; scale bars represent 100 µm. B) GAG production of MSCs encapsulated in TGF-β1-modified hydrogels after 10 and 21 d of chondrogenic differentiation, shown for total GAG (GAG/Gel) and normalized to DNA (GAG/DNA). Data are presented as means ± standard deviation ($n = 3$). (∗) Significantly different from control gels w/o TGF-β1 at the same time point ($p < 0.05$). (a) Significantly different from the TGF-β1 Medium group at the same time point ($p < 0.05$). (b) Significantly different from the 10 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (c) Significantly different from the 50 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (d) Significantly different from the 100 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (°) Significant differences of time points within a group ($p < 0.05$). Representative results of one of three independent experiments are shown.

Medium group after 21 d (Figure 7A). In contrast, the collagen signal in the 100 nM TGF-β1 group was distinctly weaker and mainly limited to pericellular regions (Figure 7A). Quantification of total collagen was well in agreement with the histological results, as the determination of collagen/DNA content showed that TGF-β1 Medium group (8.4 ± 0.2 µg/µg) and 100 nM TGF-β1 + Traut group (7.3 ± 0.8 µg/µg) produced significantly higher amounts than the 100 nM TGF-β1 group with nonthiol-functionalized growth factor (3.2 ± 1.9 µg/µg) (Figure 7B).

These results clearly demonstrated the superior effect of covalent incorporation of 100 nM TGF-β1 into the HA hydrogels compared to the noncovalent incorporation of 100 nM TGF-β1 with regard to GAG and collagen deposition. Cartilaginous ECM was formed to a much higher extent in the TGF-β1 tethered group than in the nontethered group (Figures 6 and 7).

These distinct differential biological effects were observed despite the fact that similar release profiles were detected for hydrogels with covalently and noncovalently bound TGF-β1 (Figure 5). Possible explanations include potential differences in TGF-β1 signaling. When free TGF-β1 is bound to its receptor, the protein–receptor complex can undergo endocytosis with subsequent intracellular degradation[35] leading to reduction in concentration of free protein and receptor density. In hydrogels with covalently bound TGF-β1, the protein may bind to the receptor triggering TGF-β1 signaling without consuming protein and receptor leading to prolonged signaling. Furthermore, a modulating factor contributing to the differential biological effects may be the way of growth factor immobilization and presentation, respectively, which may have been advantageously changed by the covalent binding.

In this study, PG was chosen as a structural analog for the commonly used and terminal-modified PEG derivatives. PEG hydrogels are biologically inert and need biological modification with, e.g., peptide sequences in order to allow cells to attach and recognize the hydrogel environment.[36] Additionally, they can be combined with biological macromolecules in composite hydrogels to increase their bioactivity.[8,9] PG is chemically closely related to PEG and biologically similarly inert but provides the possibility of a higher functionalization density due to side chain functionalization.[11,34] This is also true for PG-based hydrogels,[37] as we were able to show recently by utilizing thiol- and allyl-modified PG for the generation of thiol-ene clickable PG hydrogels. By replacing the thiol-modified PG component with bioactive thiol-modified HA, the deposition of cartilage-specific matrix components secreted by encapsulated MSCs, was significantly improved when compared to pure PG hydrogels.[38] Because of these recent findings we decided in the present study to combine the chondrosupportive effect of HA with the possibility to introduce precise biomimetic functionalization using acrylate-modified PG as multifunctional crosslinker at the example of TGF-β1. In comparison to previous studies investigating covalently bound TGF-β1 which were carried out using pure PEG hydrogels,[14,15] chondrogenesis of MSCs in the HA-based hydrogels presented in this study

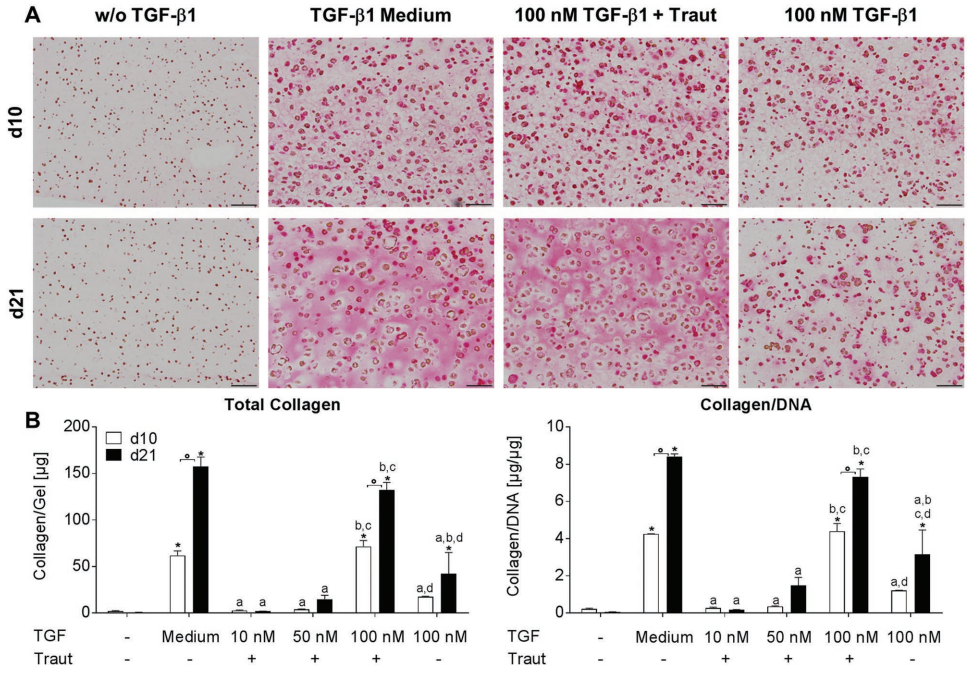

Figure 7. A) Histological stainings of TGF-β1-laden hydrogels, seeded with 20.0×10^6 MSCs mL^{-1}, after 10 and 21 d of chondrogenic differentiation. Longitudinal sections were stained for deposition of collagens with picrosirius red; scale bars represent 100 µm. B) Collagen production of MSCs encapsulated in TGF-β1-modified hydrogels after 10 and 21 d of chondrogenic differentiation, shown for total collagen (Collagen/Gel) and normalized to DNA (Collagen/DNA). Data are presented as means ± standard deviation ($n = 3$). (*) Significantly different from control gels w/o TGF-β1 at the same time point ($p < 0.05$). (a) Significantly different from the TGF-β1 Medium group at the same time point ($p < 0.05$). (b) Significantly different from the 10 nM TGF-β1 + Traut group at the same time point ($p < 0.05$), (c) significantly different from the 50 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (d) Significantly different from the 100 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (°) Significant differences of time points within a group ($p < 0.05$). Representative results of one of three independent experiments are shown.

was much more pronounced, as shown on biochemical level as well as in histological examination. This may be due to the fact that HA inherently facilitates cellular attachment and migration via the respective receptors[39,40] and showed chondrosupportive effects in previous studies.[7]

3.3.3. Immunohistochemistry

In order to examine ECM production in more detail, IHC was employed to investigate the deposition of cartilage-specific ECM components. As expected, the hydrogel constructs cultured in medium w/o TGF-β1 showed no staining for cartilage-specific ECM components, such as aggrecan or collagen II (**Figure 8**). Cells encapsulated in the 10 nM and 50 nM TGF-β1 + Traut-modified hydrogels also exhibited a very low deposition of cartilage-specific ECM components (Figure S7, Supporting Information). As already seen for deposition of GAGs, the signal for aggrecan was most pronounced in the 100 nM TGF-β1 + Traut group, exhibiting evenly distributed as well as strong pericellular signals. Aggrecan also appeared to be distributed throughout the hydrogel matrix in the TGF-β1 Medium group, while the 100 nM TGF-β1 group revealed weaker, mainly pericellular aggrecan signals (Figure 8). The most prominent staining for collagen type II was detected in the TGF-β1 Medium group, confirming the staining for total collagen by picrosirius red. The type II collagen signal in the 100 nM TGF-β1 + Traut group was also clearly detectable, but slightly dimmer and pericellularly less pronounced. In contrast to that, the group with non-covalently bound 100 nM TGF-β1 showed only few pericellular signals without deposition into the gel matrix (Figure 8). IHC for collagen type I, undesired in articular cartilage, also showed the highest amount of deposition for gels cultured in TGF-β1 containing medium, with strong pericellular signals. The collagen type I signal in the 100 nM TGF-β1 + Traut was equally distributed, but less pronounced, while the 100 nM TGF-β1 group again showed mostly weak pericelluar signals (Figure 8). For collagen X, only very minor staining was observed in all groups receiving TGF-β1 (Figure 8).

The hydrogels investigated in this study were permissive for an overall even distribution of cell-derived ECM molecules (Figures 6–8), which represents an advantageous feature of the newly developed and relatively soft materials. This is well in agreement with previous studies in which hydrogels with different stiffness were compared. It was shown for cells that were encapsulated in hydrogels with higher stiffness[7,28,29] that the deposition of ECM molecules was mainly restricted to pericellularly regions, in contrast to softer gels with a more even ECM distribution. In previous studies employing TGF-β1 covalently bound in PEG hydrogels, unfortunately mechanical properties of the hydrogels were not determined; however, the high amount of polymer applied (10 wt% PEG) suggests relatively stiff hydrogels, which in turn explains the observed uneven ECM distribution which was strongly

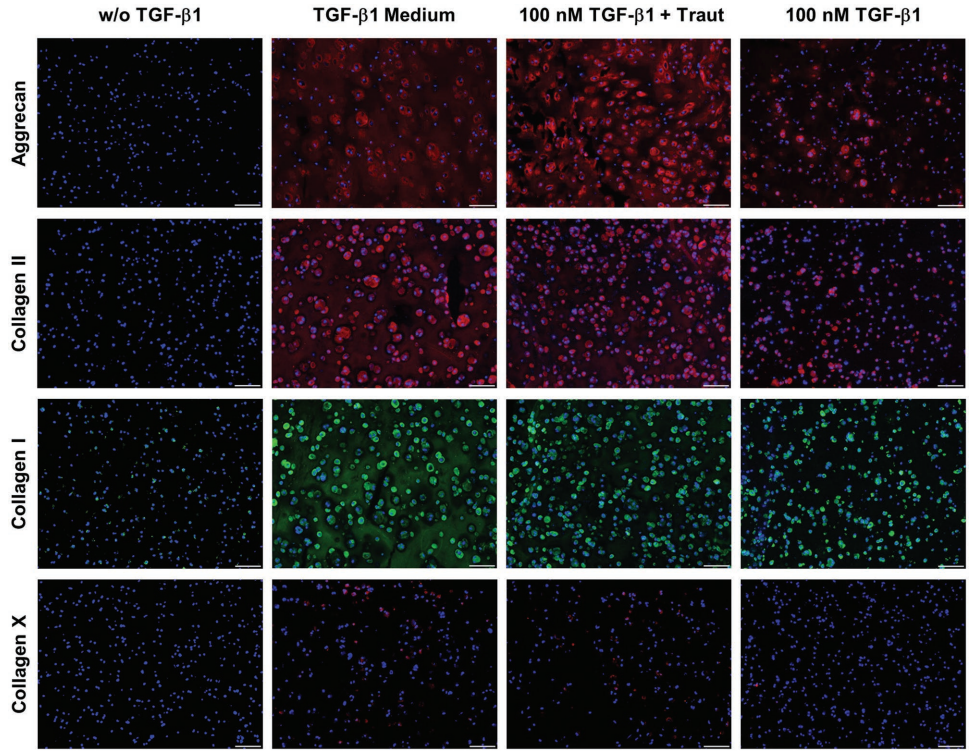

Figure 8. Immunohistochemical staining of TGF-β1-laden hydrogels, seeded with 20.0×10^6 MSCs mL^{-1}, after 21 d of chondrogenic differentiation. Longitudinal sections were either stained for aggrecan, collagen II, and collagen X (all red) or collagen I (green) to show ECM development. Nuclei (blue) were counterstained with DAPI; scale bars represent 100 µm.

pronounced in pericellular regions.[14,15] A comparative analysis focusing on the influence of HA hydrogel crosslinking density on ECM distribution showed that an increase in crosslinking density led to an overall decrease in cartilage-specific ECM production and a more limited distribution throughout the hydrogel.[29] Possible disadvantages resulting from the relative softness of hydrogels such as the ones presented here with regard to load bearing may be overcome by the utilization of reinforcement structures. Incorporation of reinforcing fibers have been shown to be advantageous for hydrogel-based cartilage engineering approaches[41] and may improve the load resistance of the hydrogels in in vivo applications.

3.3.4. Gene Expression

To further investigate possible differences between groups with different modes of TGF-β1 application, mRNA expression profiles of chondrogenic marker genes were assessed using qRT-PCR. In general, all chondrogenically differentiated gel-encapsulated MSCs showed strongly elevated expression of all analyzed genes, as compared to cells cultured in hydrogels w/o TGF-β1 (**Figure 9**). In the 100 nM TGF-β1 + Traut group, mRNA expression of the chondrogenic markers *COL2A1, ACAN,* and *SOX9* was increased significantly, compared to the 100 nM TGF-β1 group (with noncovalently bound growth factor) and the TGF-β1 Medium group (Figure 9A,B,E). Thus, the gene expression profiles of chondrogenic marker genes reflected the observations from GAG and collagen determination and immunohistochemistry and supported the beneficial effect of tethered TGF-β1 on MSC chondrogenesis compared to nontethered growth factor. *COL1A1* expression increased initially in all chondrogenically induced groups to a certain extent, but decreased as chondrogenesis progressed (Figure 9C). The assessment of the hypertrophic marker gene *COL10A1* showed also in the 100 nM TGF-β1 + Traut group a significant upregulation after 21 d, in contrast to the other chondrogenically induced groups (Figure 9D). Interestingly, this was not reflected in the immunohistochemical results, showing only very minor staining for collagen X after 21 d (Figure 8). Further investigations with an extended time frame may elucidate the long-term effects of covalently bound TGF-β1 with regard to MSC hypertrophy. In general, qRT-PCR results reflected the previous findings and showed the beneficial effect of covalently bound TGF-β1 in the 100 nM TGF-β1 group in comparison to the noncovalently bound 100 nM TGF-β1 group on MSC chondrogenesis.

4. Conclusions

PG-Acr was successfully synthesized and a hydrogel system consisting of HA-SH and PG-Acr with crosslinking via Michael addition was developed. By covalent binding of the

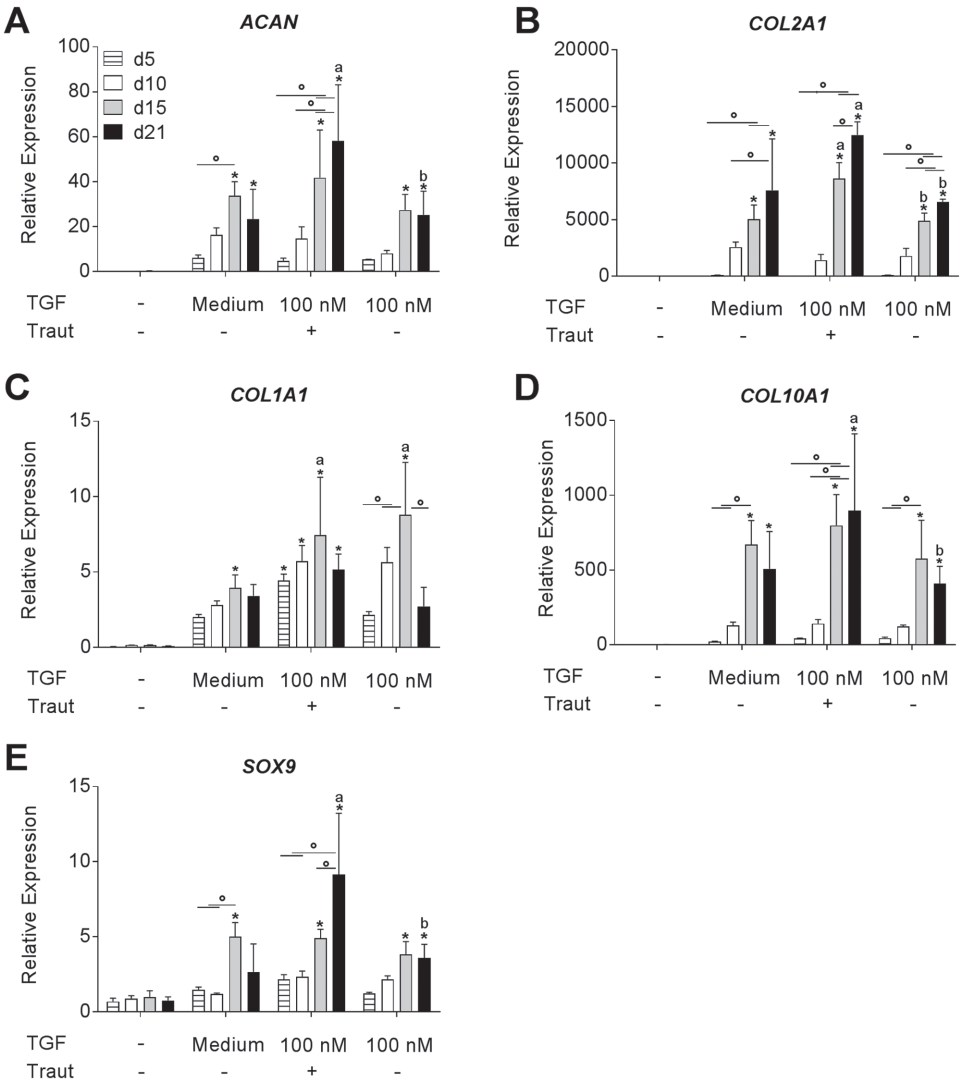

Figure 9. Time course of gene expression of MSCs encapsulated in TGF-β1-modified hydrogels after 5, 10, 15, and 21 d of chondrogenic differentiation, determined by qRT-PCR. Data are presented as means ± standard deviation ($n = 3$). (∗) Significantly different from control gels w/o TGF-β1 at the same time point ($p < 0.05$). (a) Significantly different from the TGF-β1 Medium group at the same time point ($p < 0.05$). (b) Significantly different from the 100 nM TGF-β1 + Traut group at the same time point ($p < 0.05$). (°) Significant differences of time points within a group ($p < 0.05$). Representative results of one of two independent experiments are shown.

model substance TGF-β1 via Traut's reagent to PG-Acr, the hydrogel was successfully biofunctionalized without compromising the overall gelation process and swelling behavior of the gels. With the exemplary covalent incorporation of TGF-β1, the general suitability of PG-Acr for the generation of hydrogels in cartilage tissue engineering approaches was demonstrated. Chondrogenesis of human MSCs was significantly improved in hydrogels with covalently bound TGF-β1 compared to gels without covalent incorporation of the growth factor. Possible effects on MSC hypertrophy should be further investigated. In principle, because of its multifunctionality, PG offers the opportunity to incorporate various biological cues, such as biomimetic peptides and growth factors even at the same time, into hydrogels and to thereby enhance the chondrogenic and eventually the clinical potential of the developed hydrogels.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

chondrogenesis, hyaluronic acid, mesenchymal stromal cells, poly(glycidol), TGF-β1

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