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Generation of an azide-modified extracellular matrix by adipose-derived stem cells using metabolic glycoengineering

Abstract: Natural extracellular matrix (ECM) represents an ideal biomaterial for tissue engineering and regenerative medicine approaches. For further functionalization, there is a need for specific addressable functional groups within this biomaterial. Metabolic glycoengineering (MGE) provides a technique to incorporate modified monosaccharide derivatives into the ECM during their assembly, which was shown by us earlier for the production of a modified fibroblast-derived dermal ECM. In this study, adipose-derived stem cells (ASCs) were treated with the azide-modified monosaccharide derivative 1,3,4,6-tetra-O-acetyl-N-azidoacetylgalactosamine (Ac₄GalNAz). Toxicity and viability assays after 24 h and 72 h incubation revealed high biocompatibility of Ac₄GalNAz in contact with ASCs. The successful incorporation of the functional azide groups into the glycocalyx and the ECM of the ASCs was proven by conjugation with a fluorescent dye via a copper-catalyzed click reaction. Thus, Ac₄GalNAz in combination with ASCs was confirmed to achieve an azide-modified ECM as a multifunctional biomaterial for further applications.

Keywords: Adipose-derived stem cells, clickECM, extracellular matrix, metabolic glycoengineering, azide-modified

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1 Introduction

The extracellular matrix (ECM) represents the natural environment of cells in an organism, wherein it is synthesized and assembled by tissue-specific cells. Next to native ECM, derived from decellularized mature tissues, cell-specific matrices can also be obtained from the in vitro culture of these cells. Varying with the specific tissue or cell source, the ECM contains variable amounts of collagens, other fibrous and non-fibrous proteins, proteoglycans and glycoproteins and exhibits different physical properties. Native and cell-derived ECMs are used and studied in a variety of applications as potential biomaterials such as cell-influencing coatings [1, 2], hybrid scaffolding materials for tissue engineering [3, 4], and bioinks [5, 6]. A wide range of potential applications requires the modification of the ECM with specific addressable functional groups. Chemical modification of ECM compounds is challenging as it might affect matrix integrity. To address these issues, metabolic glycoengineering (MGE) represents a promising tool. This method is based on the incorporation of chemically modified monosaccharide derivatives into the natural intra- and extracellular oligosaccharide structures of the cell by its natural metabolic pathways [7, 8]. These functionalities can subsequently be addressed by bioorthogonal chemical ligation reactions [9, 10] for tuning chemical and physical properties and for visualization of glycoconjugates. Previously, we employed MGE with the azide-modified monosaccharide derivative 1,3,4,6-tetra-O-acetyl-N-azidoacetylgalactosamine (Ac₄GalNAz) to generate a human fibroblast-derived dermal ECM containing azide groups [11] that can be addressed by copper-catalyzed azide-alkyne cycloaddition [12, 13]. For this functionalized ECM (clickECM) we could show that the composition and functionality of the ECM was not affected by the MGE procedure and the modification with azide groups [11]. Later, also Gutmann et al. reported a similar approach using the corresponding azide-modified glucosamine derivative [14].

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In this study, we aimed to prove whether the glycocalyx and ECM of adipose-derived stem cells (ASCs) can be modified with azide groups using Ac₄GalNAz in MGE (Figure 1). Generation of an azide-modified stem cell ECM would be very promising for a wide range of applications especially in tissue engineering and regenerative medicine by representing a stem cell niche material with tunable chemical and physical properties. Such a unique biomaterial cannot be achieved by decellularization of tissues, as the stem cell niches are very small in vivo and integrated in matured neighboring tissues. Therefore, azide-modified ECM of ASCs provides multiple opportunities of further functionalization and application.

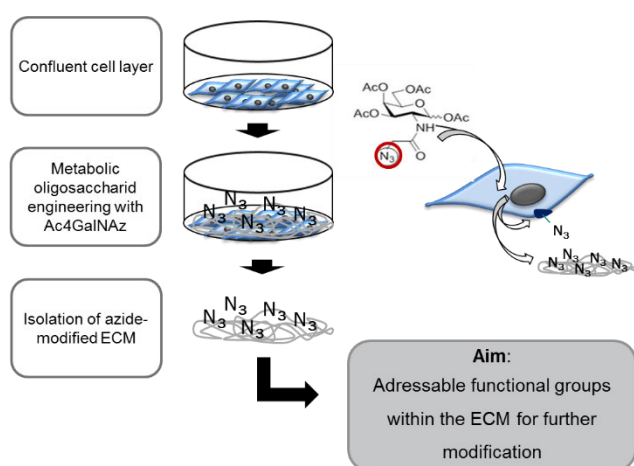


Figure 1: Schematic overview of the metabolic glycoengineering process with ASCs and Ac₄GalNAz. Cells were grown to confluency followed by the addition of Ac₄GalNAz. The incorporation of azide-groups into the glycocalyx and ECM occurs by MGE. Subsequently the azide-modified ECM was isolated by lysis of the cells. Generated azide-modified ECM could prospectively be used for further applications such as specific modifications of the ECM with molecules and for the use in tissue engineering and bioprinting approaches. (Modified after Ruff et al. [11])

2 Materials and Methods

2.1 Isolation and expansion of ASCs

ASCs were isolated from human tissue samples obtained from patients undergoing plastic surgery (Dr. Ziegler; Klinik Charlotenhhaus, Stuttgart, Germany) as described before [15]. All research was carried out in accordance with the rules for

investigation of human subjects as defined in the Declaration of Helsinki. Patients provided written agreement in compliance with the Landesärztekammer Baden-Württemberg (F-2012- 078, for normal skin from elective surgeries). ASCs were initially seeded at a density of 5×10^3 cells cm^{-2} in serum-free MSC growth medium (PELOBiotech, #PB-C-MH-675-0511-XF) containing 5 % human platelet lysate.

2.2 Biocompatibility assay

The biocompatibility of Ac₄GalNAz was evaluated by a lactate dehydrogenase (LDH) assay (TaKaRa Bio Inc. #630117) and a resazurin assay (Sigma Aldrich, #R7017). ASCs were treated with 100 μM Ac₄GalNAz or phosphate buffered saline (PBS) for 24 h and 72 h. LDH assay was performed according to manufacturer's protocol with cell culture supernatant. For the resazurin assay, culture medium was changed to medium with resazurin salt (11 $\mu\text{g}/\text{mL}$) and incubated for 3 h at 37 °C and 5 % CO₂.

2.3 Detection of incorporated azide groups

Incorporated azide-groups were detected via copper-catalyzed azide-alkyne cycloaddition using an alkyne-linked fluorophore as described by Ruff et al. [11]. Isolated paraformaldehyde-fixed ECM was stained according to the manufacturer's instructions. Cell nuclei were counterstained with 1 $\mu\text{g}/\text{mL}$ 4',6-diamidino-2-phenylindole (DAPI, Serva Electrophoresis) in PBS for 10 min at RT. Fluorescence images (z-stacks) were taken using a Zeiss Axio Observer.

2.4 Statistics

All experiments were performed with cells from three different biological donors. Data was compared by a one-way analysis of variance and a Tukey post-hoc test using OriginPro 2018b.

3 Results and Discussion

Biocompatibility of Ac₄GalNAz was proven by LDH and resazurin assay (Figure 2). Control groups (Ctr) were incubated with PBS and absorption values were normalized to the control groups. LDH assay revealed no cytotoxic effects of Ac₄GalNAz after 24 h and 72 h.

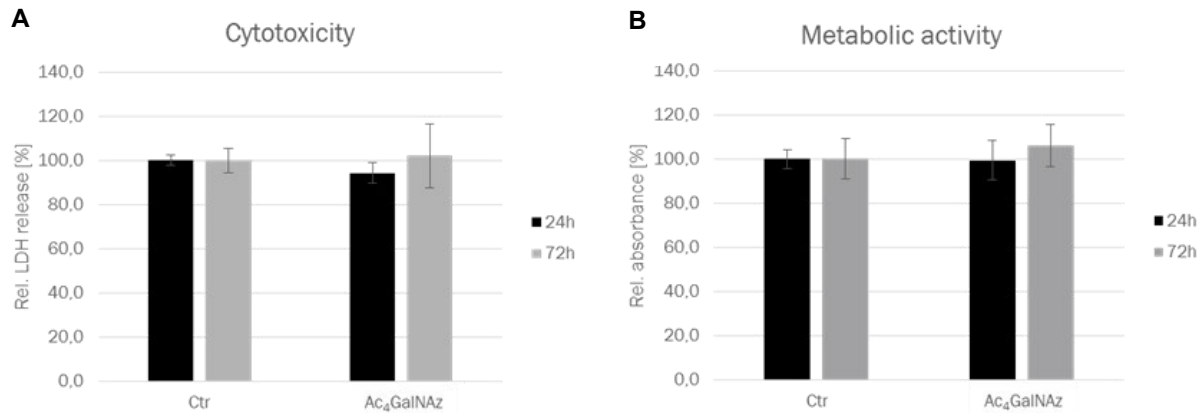


Figure 2: Biocompatibility of Ac₄GalNAz in ACSs. ASCs were incubated with 100 μ M Ac₄GalNAz for 24 h and 72 h. Controls were treated with PBS. A: LDH Assay revealed no significant influence of azide-modified monosaccharide on cell death after 24 h and 72 h. B: Resazurin assay showed no significant influence of azide-modified monosaccharide on metabolic activity after 24 h and 72 h.

Metabolization of resazurin salt was comparable for cells treated with Ac₄GalNAz to the control group. Thus, resazurin assay revealed no significant influence of Ac₄GalNAz on metabolic activity of ASCs. These results are in line with previous studies using fibroblasts [11].

ECM of ASCs can be successfully isolated and harvested as thin sheet of protein network (Fig. 3 A;i) or left as thin gel-like film on the bottom of the petri dish (Figure. 3 A;ii). Incorporation of azide-groups into the glycocalyx and ECM of ASCs was proven by reaction with fluorophore-linked alkyne. In the untreated control group, no specific fluorescence staining was observed (Figure 3B). The cells treated with Ac₄GalNAz exhibited a high specific fluorescence staining of

the incorporated azide-groups. It was shown that azide groups were successfully incorporated into the glycocalyx of the cells. Moreover, for ECM isolated from ASCs and thereafter treated with Ac₄GalNAz, showed a specific fluorescence of stained azide groups. This confirmed the incorporation of Ac₄GalNAz in the stem cell ECM. The azide groups were ubiquitously distributed in the glycocalyx and the ECM, comparable to the findings of Ruff et al. [11]

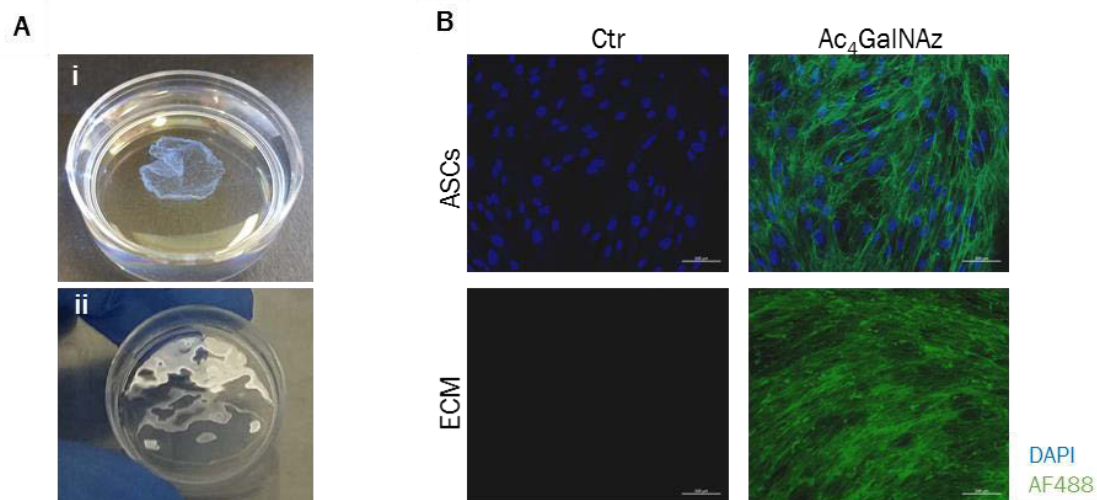


Figure 3: Characterization of ECM as biomaterial. A: Macroscopic pictures of isolated ECM. i: Isolated ECM floating in PBS. ECM can be isolated and collected as a gel-like sheet of a collagen network. ii: Isolated ECM on the bottom of a petri dish. After cell lysis and washing steps, the collagen network appears as a gel-like coating on the bottom of a petri dish ($d=35$ mm) **B: Fluorescence staining of the incorporated Ac₄GalNAz in ASCs and cell-derived ECM.** Staining of the azide-groups shows the successful incorporation of azide groups into the glycocalyx and ECM of the ASCs. Negative controls (Ctr.) exhibit no fluorescence staining. Cell nuclei are counterstained with DAPI. (Scale bar: 100 μ m)

4 Conclusion

No negative impact on cell survival and metabolic activity of ASCs was detectable. Further, it was shown that stem cell ECM of ASCs can be successfully modified with azide groups, which can be further addressed by alkyne-groups via copper-catalyzed azide-alkyne cycloaddition. Based on these results, Ac₄GalNAz may be considered a suitable azide-modified monosaccharide derivative to be used in MGE with ASCs. Thereby, this approach opens up a wide field of medical and material applications of ASC-derived ECM by yielding a multifunctional biomaterial with tuneable chemical and physical properties.

Author Statement

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Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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