Introduction

The extracellular matrix (ECM) is a complex 3D-network that is secreted by various cell types. The ECM has different essential roles in regulating the function, development and homeostasis of eukaryotic cells. It provides mechanical support, regulates the abundance of signaling molecules (e.g. growth factors) and receptors as well as pH and hydration status. The extracellular matrix is primarily composed of water, proteins and polysaccharides which shows exquisite tissue specificity, as a result of the unique ECM composition that is generated through a dynamic biochemical and biomechanical play between the tissue-resident cells. Cell derived matrices (CDM) have been recently attracted attention as biocompatible scaffold material for skeletal tissue engineering and cardiovascular/vascular tissue engineering based on the possibility to engineer these CDM de novo based on cell source and culture methods [1].

Materials & Results

The fluorescence band in lane 1 shows the specific N4-reactivity of the DBCO-eGFP. The different lanes show that the click reaction took place within 5 min and coupling efficiency increased with time.

Extracellular matrix (ECM) generation

The ECM was produced by NIH3T3 fibroblasts over 9 days. Cells were seeded on crosslinked gelatin-coated cover slides and treated for 9 days with the addition of 25 µM sodium ascorbate and with 50 µM of Ac6GlcNAz. After 9 days the ECMs were decellularized using Cukierman H2O extraction protocol [4].

Confocal laser scanning microscopy (CLSM) was performed with the ECM after 9 days. The ECM was decorated with Sulfo-Cys-alkyne / DBCO-sulfo-Cys [3] and native structure was stained using Anti-Fibronectin and Anti-IGF Alexa Fluor 488 antibody. Fluorescence images were taken using a Leica AOB5 SP2 (Leica microsystem).

References