ADVANCED HEALTHCARE MATERIALS

Supporting Information

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A Versatile Biosynthetic Hydrogel Platform for Engineering of Tissue Analogues

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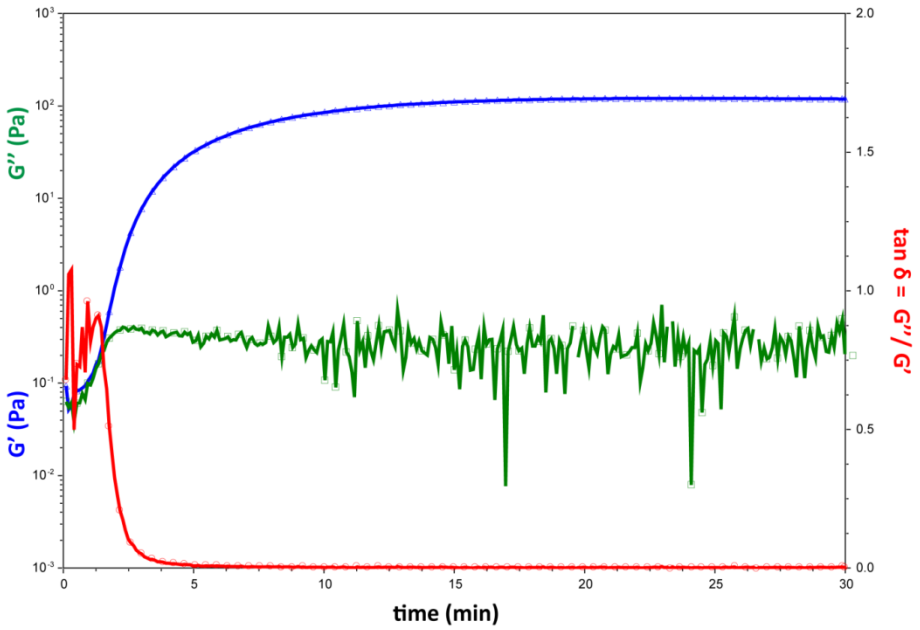
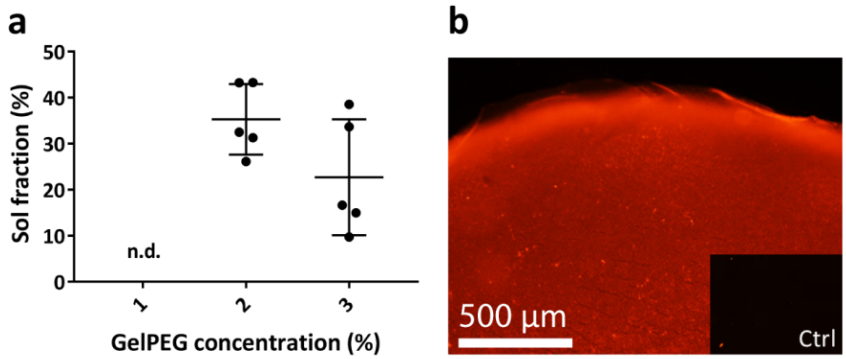
Supporting Information

Supplementary comment 1.

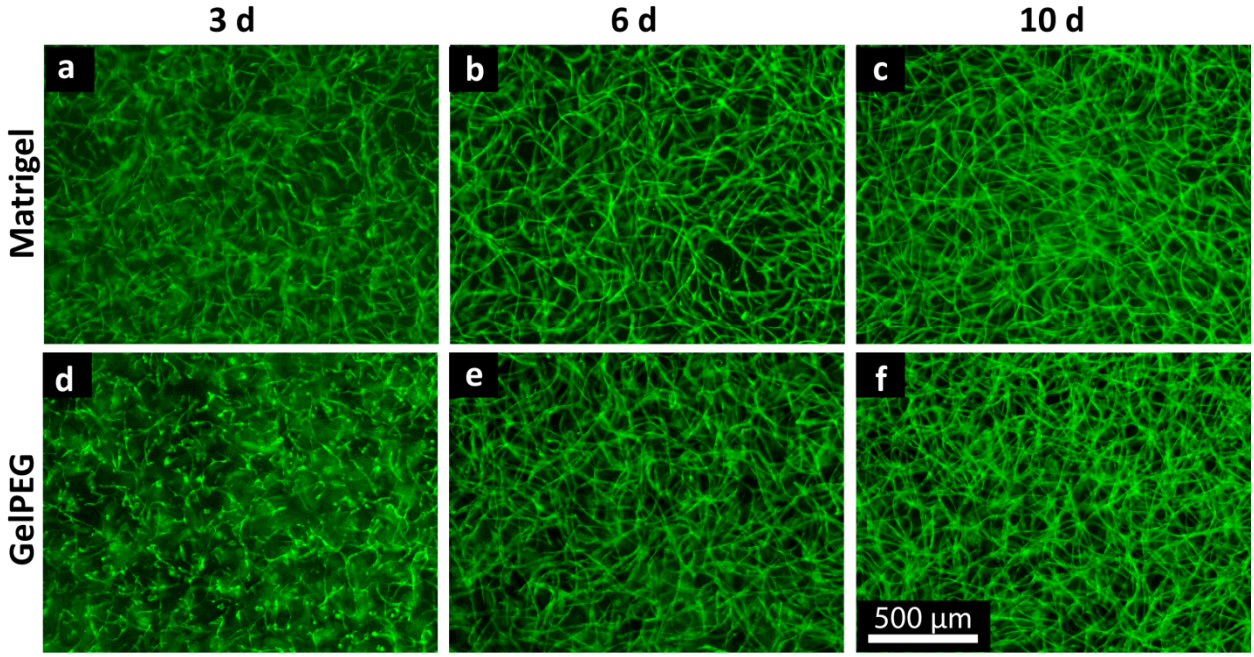
We hypothesized, that while the amino acid sequence containing a glutamine is a very specific sequence that the lysine (Lys) containing sequence is more generic. Indeed, hydrogels did not form in the absence of PEG-Gln when PEG-Lys was combined with gelatin. To exclude the formation of intramolecular crosslinks, pure gelatin was crosslinked at a concentration of 10% w/v with FXIIIa. Under present crosslinking conditions, pure gelatin did not form a hydrogel and the polymer solution remained at a low viscosity (data not shown), demonstrating the need for the substrate-specific Gln-sequence for FXIIIa mediated crosslinking.



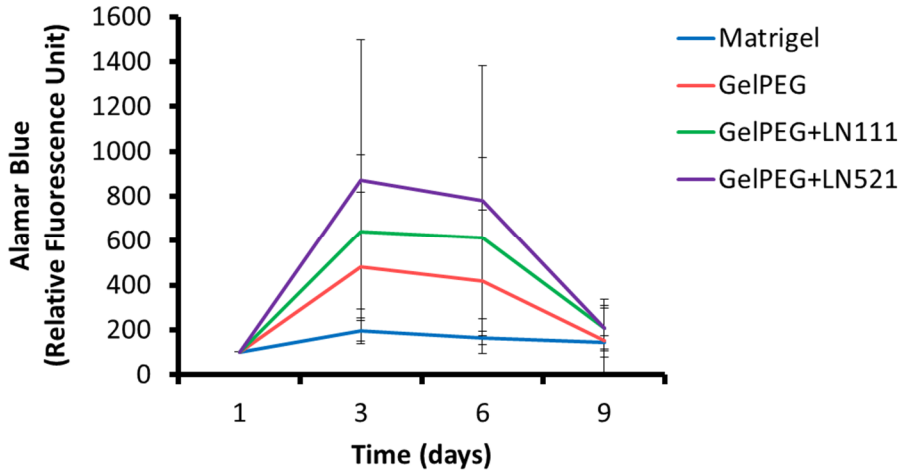
Supplementary Figure 1. Hydrogel formation of gelatin and PEG mediated by FXIIIa. The point of gelation occurred at about 2 minutes and the crosslinking was completed after about 15 minutes, when G' reached a plateau. Depicted data are from a representative measurement; *n*= 3 independent experiments



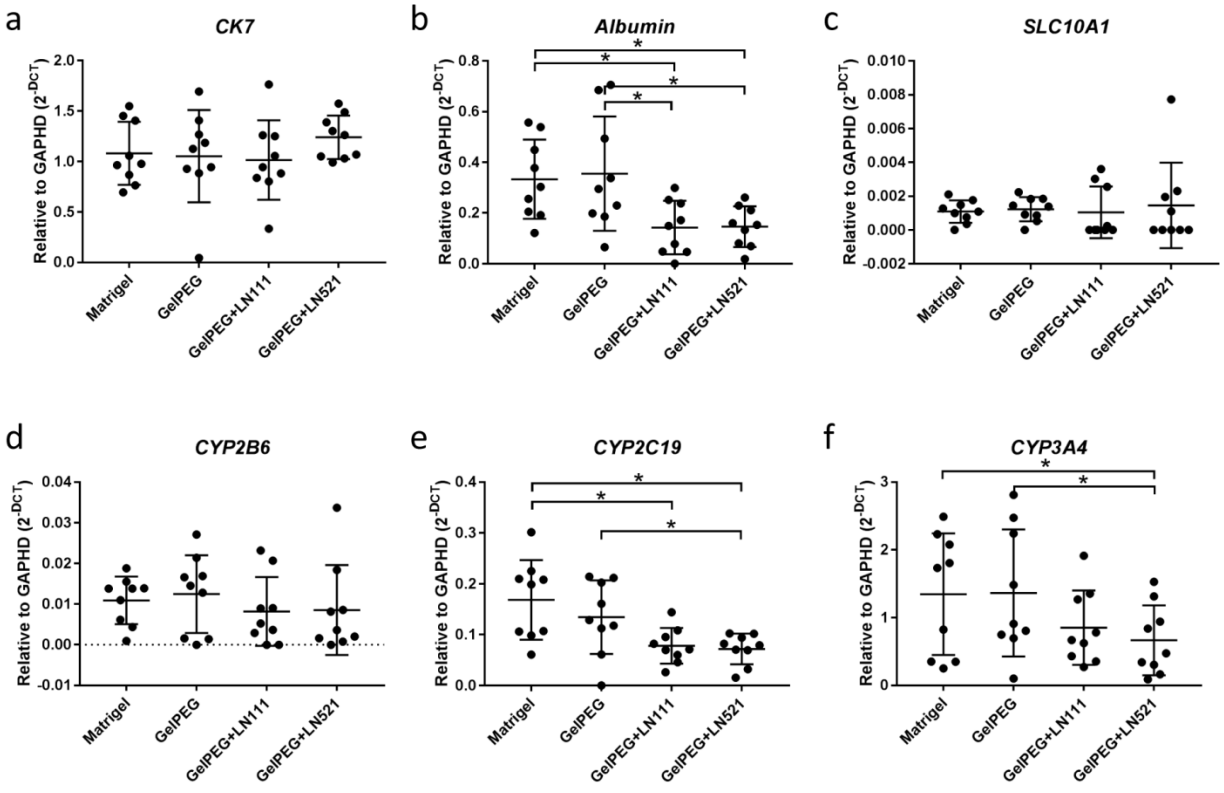
Supplementary Figure 2. Hydrogel sol fraction and LN binding in gelPEG hydrogel networks. **a** Sol fraction in dependence of gelPEG concentration. **b** Anti-LN-subunit α5 staining of LN521-laden 3% w/v gelPEG hydrogel compared to unloaded hydrogel (insert) after 1 d swelling in TBS. Data is depicted as mean + SD, *n*= 5



Supplementary Figure 3. Pre-vascular network formation in GFP-ECFC and MSC co-cultures under vasculogenic culture conditions. **a-c** GFP-ECFCs in Matrigel form vascular-like networks on day 3 which are remodeled up to day 10. **d-f** GFP-ECFCs in gelPEG hydrogels form vascular-like structures with an initial temporal delay, resulting in comparable networks to Matrigel on day 6 and 10. (widefield fluorescence, *N*= 3, *n*= 3*)*



Supplementary Figure 4. Metabolic activity of liver organoids over culture time in Matrigel and gelPEG-based hydrogels. Liver organoids exhibited an increase in metabolic activity from day 1 to 3, which gradually decreases over culture time, resulting in comparable activity levels as liver organoids cultured in Matrigel after 9 days culture. Data is depicted as mean +/- SD*; N*= 3, *n*= 5



Supplementary Figure 5. Gene expression levels of liver organoids that were cultured for 9 days in Matrigel and gelPEG-based hydrogels. **a** The cytokeratin *KRT7 (CK7)* was equally expressed in all

hydrogels. **b** Albumin expression was decreased in LN-laden gelPEG hydrogels. **c** *SLC10A1,* encoding a liver-specific sodium/bile acid cotransporter, was comparably expressed in all hydrogel compositions**.** The cytochrome family of enzymes **d** CYP2B6 **e** CYP2C19 and **f** CYP3A4 were comparably expressed in Matrigel and gelPEG, whereas LN-laden hydrogels showed partially lower expression levels. Data is depicted as mean + SD*; N*= 3, *n*= 3

Tables

Supplementary table 1: Primer sets used for qPCR analysis

Human gene	**Forward primer**	**Reverse primer**
Pre-vascularized bone:		
PECAM1	GCAGTGGTTATCATCGGAGTG	TCGTTGTTGGAGTTCAGAAGTG
CDH5	AAGCAGGCCAGGTATGAGAT	TGTGTACTTGGTCTGGGTGAAG
CSPG4	GAAGGAGGACGGACCTCAAG	GATCAGCTGCTCTTCCACCATT
ACTA2	ATGCCATCATGCGTCTGGAT	ACGCTCAGCAGTAGTAACGA
BGLAP	CCTCACACTCCTCGCCCTAT	GCTTGGACACAAAGGCTGCAC
SPP1	GCCGAGGTGATAGTGTGGTT	GTGGGTTTCAGCACTCTGGT
GAPDH	CAACGGATTTGGTCGTATTGGG	TGCCATGGGTGGAATCATATTGG
Liver organoids:		
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
KRT7	GGACATCGAGATCGCCACCT	ACCGCCACTGCTACTGCCA
CYP2C19	GGGACAGAGACAACAAGCA	CCTGGACTTTAGCTGTGACC
ALB	GTTCGTTACACCAAGAAAGTACC	GACCACGGATAGATAGTCTTCTG
SLC10A1	GATATCACTGGTGGTTCTC	ATCATCCCTCCCTTGATGAC
CYP3A4	CACAGGCTGTTGACCATCAT	TTTTGTCCTATAAGGGCTTT
CYP2B6	CTACCAAGATCAAGAGTTCCTG	ATTTCAAGAAGCCAGAGAAGAG