Tailored hyaluronic acid- and fibrinogen-based hydrogels enable three-dimensional co-cultivation of HepG2 and LX-2 cells to create functional, liver-like tissue

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BACKGROUND

- Liver extracellular matrix ECM is primarily composed of collagens, glycoproteins and proteoglycans like hyaluronic acid[1].
- The hepatocellular carcinoma cell line HepG2 display most genotypic features of primary hepatocytes, but partly lower enzyme activity[2].
- Hepatic stellate cells such as the immortalized cell line LX-2 interact with hepatocytes via cytokine, chemokine and growth factor secretion and can promote hepatocyte proliferation[3].

AIM

- Effect of co-cultivation of LX-2 cells with HepG2 cells in hydrogels
- Tailor hydrogels towards co-cultivation of HepG2 and LX-2 cells
- Assess cell viability, functionality and morphology

METHODS

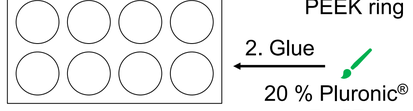
Commercial hydrogel kits:

Component	HyStem®-C 1 %	HyStem®-C 1.25 %	HyStem®-HP 1 %	HyStem®-HP 1.25 %
Glycosil® Thiol-modified hyaluronan	1 %	1.25 %	-	-
Heprasil® Thiol-modified hyaluronan and thiol-modified heparin	-	-	1 %	1.25 %
Gelin-S® Thiol-modified gelatin	1 %	1.25 %	1 %	1.25 %
Extralink® Thiol-reactive crosslinker PEGDA	3 %			

Tailored hydrogels:

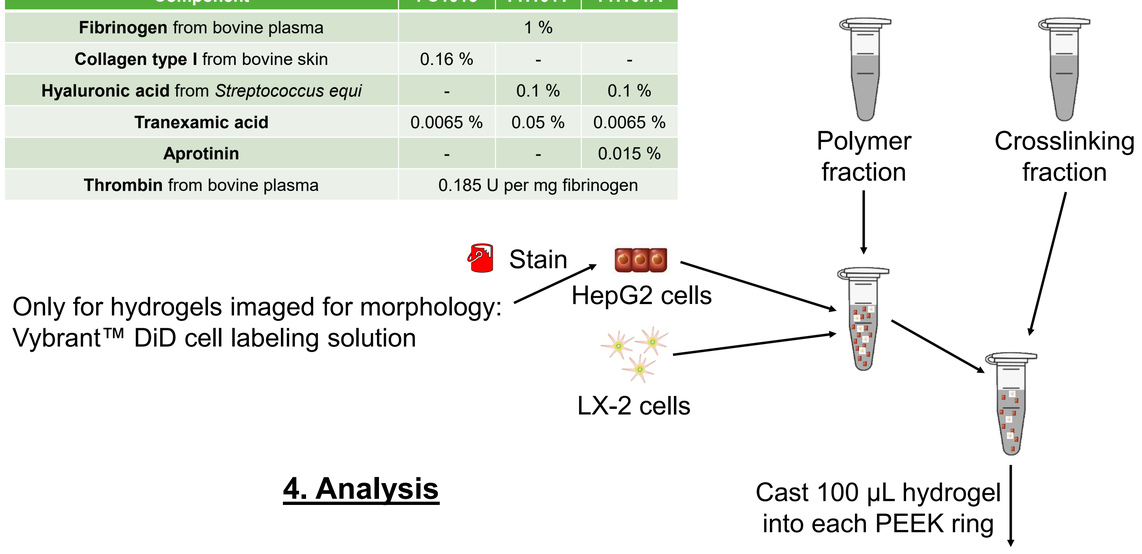
Component	FC1016	FH101T	FH101A
Fibrinogen from bovine plasma	1 %		
Collagen type I from bovine skin	0.16 %	-	-
Hyaluronic acid from *Streptococcus equi*	-	0.1 %	0.1 %
Tranexamic acid	0.0065 %	0.05 %	0.0065 %
Aprotinin	-	-	0.015 %
Thrombin from bovine plasma	0.185 U per mg fibrinogen		

1. Well plate preparation



2. Hydrogel preparation

Mix all hydrogel components



3. Cultivation

- After crosslinking: Add 1 – 2 mL medium (RPMI 1640 + 10 % FCS)
- After 1 - 2 days: Remove PEEK rings
- Every 2 – 4 days: Media change and sample collection

4. Analysis

A Live dead staining (calcein/PI/Hoechst) → Count dead and total cells with ImageJ plugin StarDist

B Human albumin ELISA with medium supernatants

C Actin staining with Alexa Fluor™ 488 phalloidin → Two photon laser scanning microscopy (z-stacks) → maximum intensity projections

RESULTS

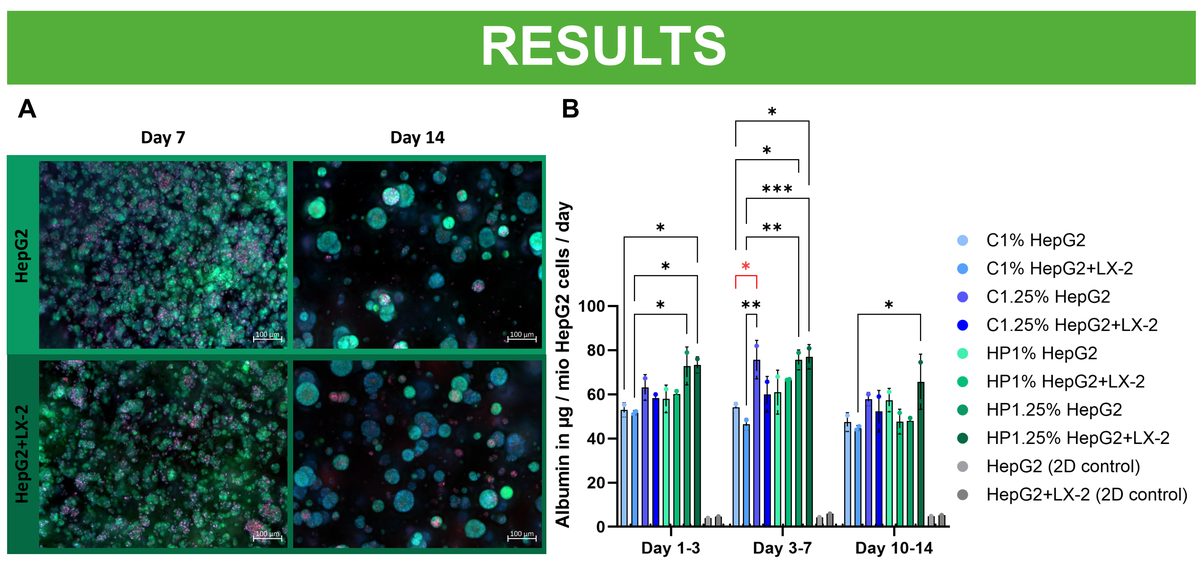


Figure 1. Viability and albumin secretion of 3 mio HepG2/mL (and 0.3 mio LX-2/mL) cultivated in HyStem®-C and HyStem®-HP hydrogels. **A**: Live/dead staining of cells cultivated in HyStem-HP 1.25 % with calcein (live; green), PI (dead; red) and Hoechst (blue; nuclei). **B**: Albumin secretion quantified from cultivation medium.

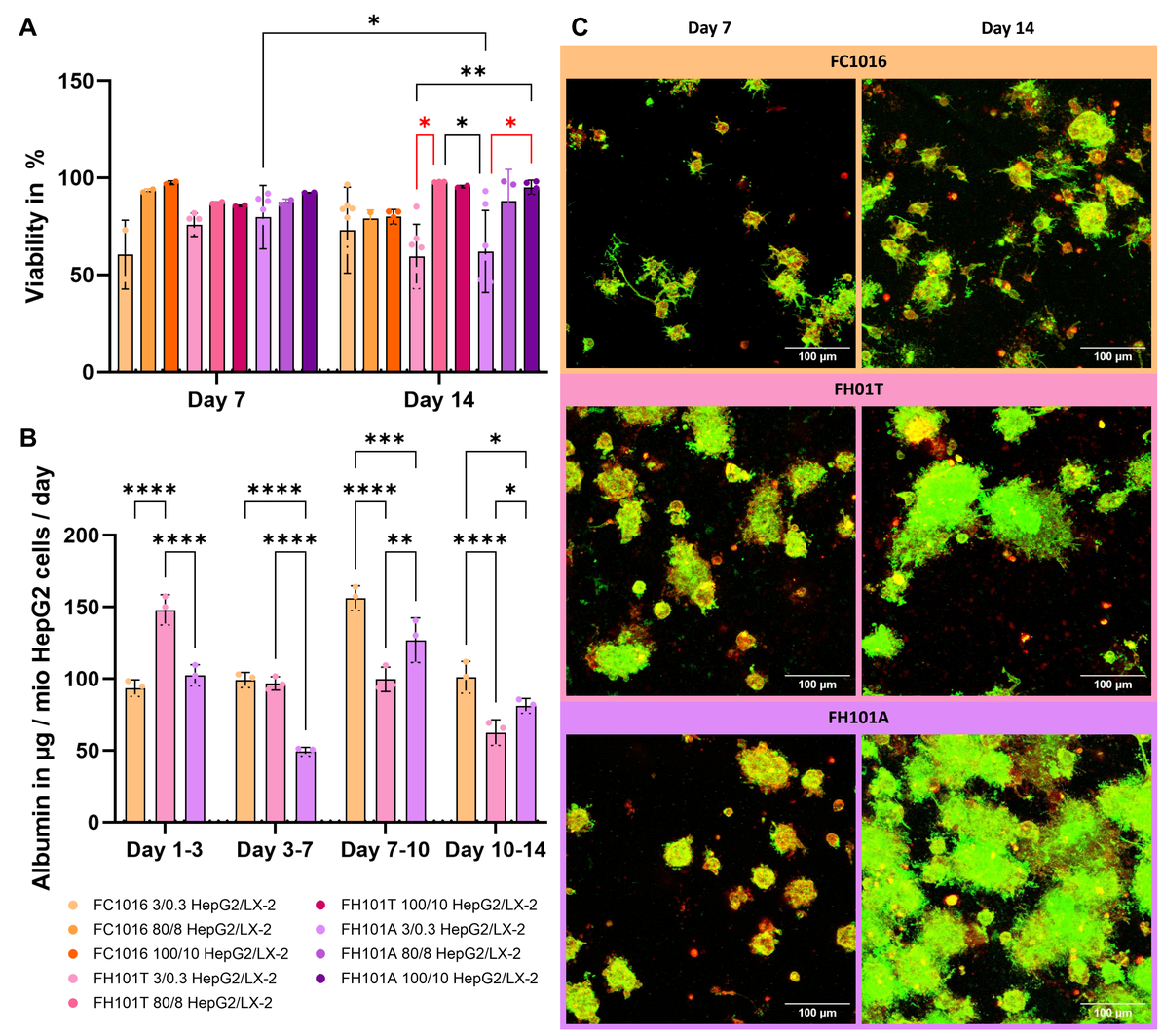


Figure 2. Viability, albumin secretion and morphology of HepG2 and LX-2 cells co-cultivated in tailored hyaluronic acid- and fibrinogen-based hydrogels. **A**: Cell viability determined using live/dead staining. **B**: Albumin secretion quantified from cultivation medium. **C**: Two photon laser scanning microscopy images of HepG2 (DiD; red) and LX-2 cells, both immunostained for actin (green). **A**: 3, 80 and 100 HepG2/mL and 0.3, 8 and 10 LX-2/mL (ratio 1:10). **B – C**: 3 mio HepG2/mL and 0.3 mio LX-2/mL.

CONCLUSIONS

Commercial hydrogel kits:

- No significant effect of co-cultivating LX-2 cells with HepG2 cells
- Highest albumin secretion rates in HyStem®-HP 1.25 %

Tailored hydrogels:

- Highest viability at physiological cell concentrations
- Different timelines of albumin secretion
- Two times higher albumin secretion compared to commercial hydrogel kits
- Highest cell spreading in FH101A

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