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COMPARISON OF DIFFERENT BIOINKS FOR LONG-TIME PRINTING

INTRODUCTION

Bioprinting is a suitable technique to generate living tissue. Therefore, the development of bioinks that support growth and organisation of cells as well as construct shape and stability is an active research field. Many bioinks are commercially available now. However, the 3D printing of cells is still challenging. Especially limited viability of cells during long printing time, cell sedimentation, and clumping of the ink make it difficult to successfully print biological structures. In this study, we compared a range of different hydrogel bioinks for their capacity to support cell viability in long printing processes and to prevent cell settlement in the cartridge.

RESULTS

We observed good printability of all bioinks tested, however significant differences concerning cell sedimentation in the cartridge and printing accuracy of the construct appeared. Especially, low viscose inks showed a dramatic change in cell numbers, as expected.

Table1\List of bioinks and printing processing parameters

	Alginate	Alginate-CMC	Alginate-Gelatin	Gelatin-Gelatin	Gelatin 2%-Collagen	Gelatin 5%-Collagen
Printing temperature	22,5°C	22,5°C	26,4°C	22,5°C	22,5°C	22,5°C
Polymer concentration	3,5% Alginate, 0,2% Calcium chloride	8% Alginate, 4% Carboxymethyl-cellulose	8% Alginate, 20 % Gelatin (300 Bloom)	Gelatin (90-110 Bloom), Gelatin (300 Bloom), 1:1	2 % Gelatin, 2 mg/ml Collagen	5 % Gelatin, 2 mg/ml Collagen
Extrusion pressure	65 kPa	115 kPa	115 kPa	50 kPa	5-10 kPa	30 kPa

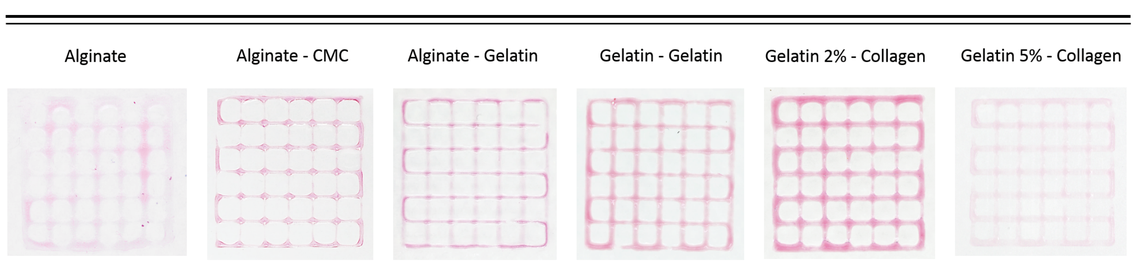

Figure 1\Makroscopic appearence of printed two-layer meshes using different hydrogel bioinks. The inks were stained with food color for better visualisation.

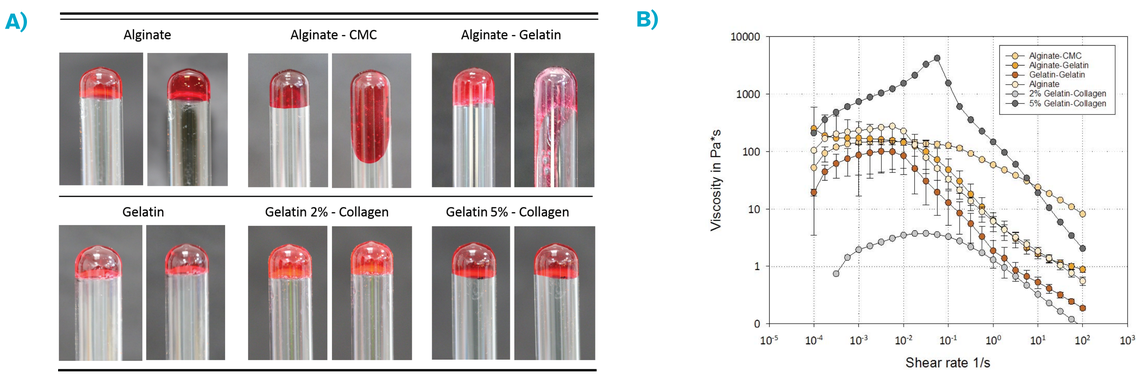

Figure 2\Viscosity of different bio-inks A) in glass tubes immediately (left) and after 5 min (right) and B) under different shear rates.

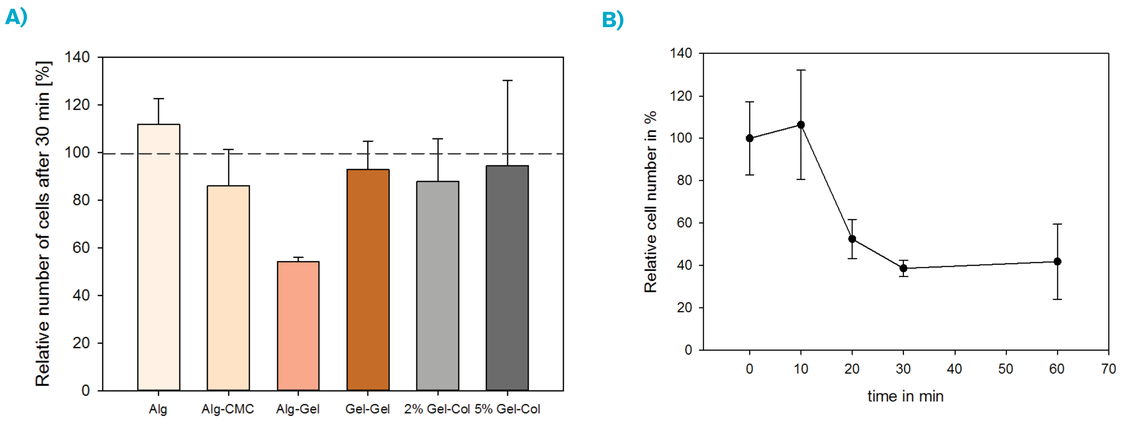

Figure 3\Cell sedimentation. A) Relative number of cells in 1 ml bioink after 30 min in the cartridge (left to right: Alginate, Alginate-Carboxymethylcellulose, Alginate-Gelatin, Gelatin, 2% Gelatin-Collagen, 5% Gelatin-Collagen). B) Changes in the number of cells over time in Alginate-Gelatin-ink.

Printing accuracy was high for higher viscose material. Especially the mixture of Alginate and Carboxymethylcellulose performed very well. However, cell viability was significantly affected by this bioink.

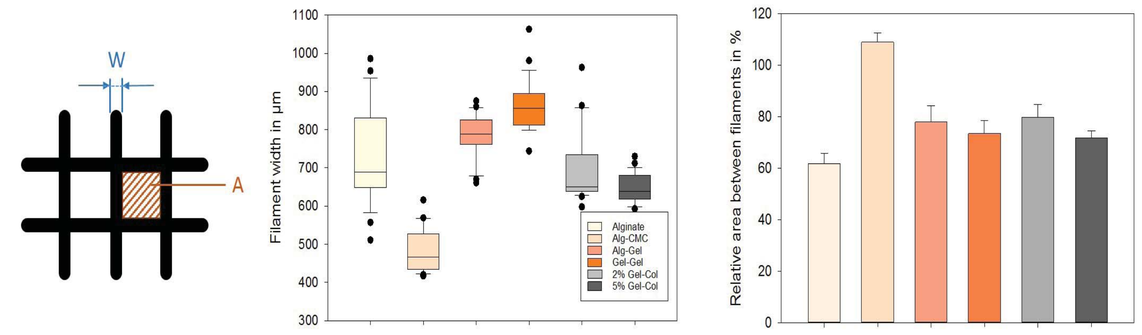

Figure 4\Printing accuracy. Filament width (inner diameter of the needle: 400 µm) and relative area between filaments of one or two-layer constructs printed with different bioinks.

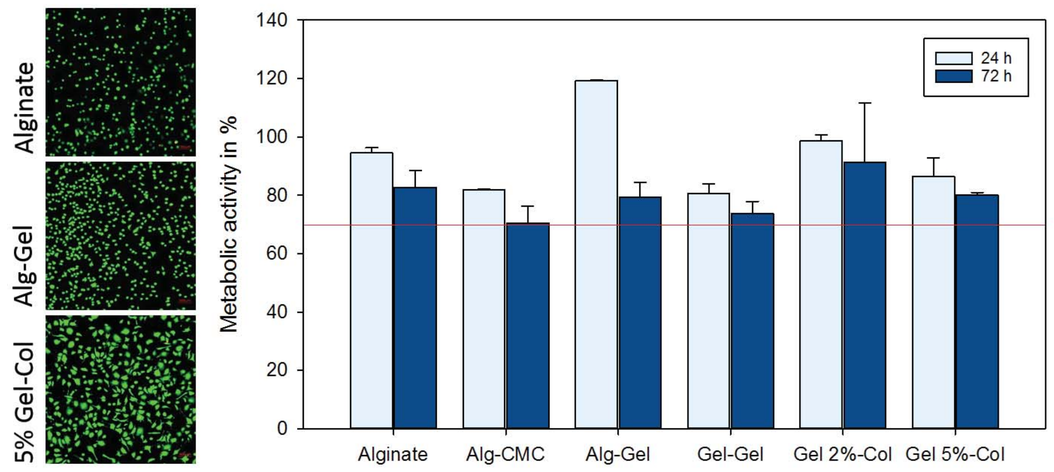

Figure 5\Live-dead staining after printing (left) and metabolic activity of cells after one and 3 days cultiviation in different bioinks (right).

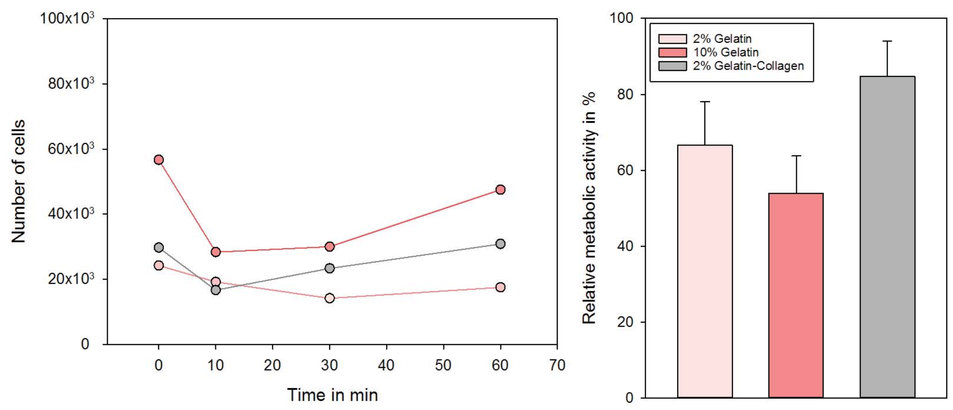

Figure 6\Effect of collagen additive on cell sedimentation and metabolic activity of cells after 3 days of cultiviation.

Bioinks that contain an increased gelatine content provided constant cell numbers, but displayed impaired cell viability over time. By adding a small amount of fibrils using a neutralized collagen solution we were able to improve cell viability and to prevent sedimentation in the cartridge.

CONCLUSIONS

The suitability of a bioink is not only dependent on printability and mechanical properties, but also on the duration time the construct needs until it is printed. Long printing times negatively affect cell sedimentation and viability in various bioinks.

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ACKNOWLEDGEMENT
The research project MF140154 was partly funded in the framework of the funding program "INNO-KOM-OST" of the Federal Ministry of Economics and Energy based on a resolution of the German Bundestag. We would like to thank for the support granted.

Supported by:
Federal Ministry for Economic Affairs and Energy
on the basis of a decision by the German Bundestag

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