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# The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability

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ABSTRACT

In the present study, we report on the combined efforts of material chemistry, engineering and biology as a systemic approach for the fabrication of high viability 3D printed macroporous gelatin methacrylamide constructs. First, we propose the use and optimization of VA-086 as a photo-initiator with enhanced biocompatibility compared to the conventional Irgacure 2959. Second, a parametric study on the printing of gelatins was performed in order to characterize and compare construct architectures. Hereby, the influence of the hydrogel building block concentration, the printing temperature, the printing pressure, the printing speed, and the cell density were analyzed in depth. As a result, scaffolds could be designed having a 100% interconnected pore network in the gelatin concentration range of 10–20 w/v%. In the last part, the fabrication of cell-laden scaffolds was studied, whereby the application for tissue engineering was tested by encapsulation of the hepatocarcinoma cell line (HepG2). Printing pressure and needle shape was revealed to impact the overall cell viability. Mechanically stable cell-laden gelatin methacrylamide scaffolds with high cell viability (>97%) could be printed.



## 1. Introduction

In recent years, cell culture substrates exhibiting high biocompatibility have been extensively revised. A steady paradigm shift from conventional 2D cell culture models toward 3D microenvironments has been observed [1]. Cell responses due to the microenvironment (mechanical and chemical) tend to differ between both models [2]. Developments in (rapid) prototyping techniques, which were already well established in other industries (e.g. automotive industry), have enabled researchers to expand their *in vitro* tissue models toward highly controlled three-dimensional (porous) scaffold architectures [3–8]. Three-dimensional porous scaffold designs allow for improved cell–cell contact, cell–matrix interactions, and increased cell densities [3,9]. Furthermore, more efficient blood vessel ingrowth and enhanced oxygen, nutrient and waste diffusion are plausible.

Post-fabrication cell seeding benefits from these advantages but is often correlated with insufficient seeding efficiency and/or a non-uniform cell distribution [10,11]. To tackle these problems, combining prototyping techniques with high-water content polymers and cell encapsulation strategies can serve as an alternative. Nevertheless, the generation of cell-laden prototyped scaffolds remains challenging and is mainly limited to hydrogel processing [3]. During processing of cell-laden hydrogel mixtures two major disadvantages can be described. First, the loss of cell viability due to dispensing pressure and nozzle diameter, which has been described by Chang and Sun [12] for the encapsulation of HepG2 during alginate plotting. Second, hydrogel mechanically stable construct built-up without internal pore collapse remains the main challenge, even in the absence of cells [13–15]. Generally, the latter is tackled by blending gelatins with other hydrogel materials, and/or co-deposition of thermoplastic materials [6,16–21]. For instance, Schuurman et al. [22] recently opted for blending of hyaluronic acid and/or co-deposition of poly-ε-caprolactone in order to be able to process 20 w/v% gelatin hydrogels without internal collapse. Another approach consists of printing sacrificial material at the future pore locations [7].

Additionally, enhanced control over matrix stiffness and liquid flow (e.g. shear stress responses) are reported applying 3D culture models [23]. In this manner, close interactions between biological, chemical and engineering cues are important in the feedback process for the generation of, for example, drug screening tools or implantable devices (Fig. 1). For example, Chang et al. [12]

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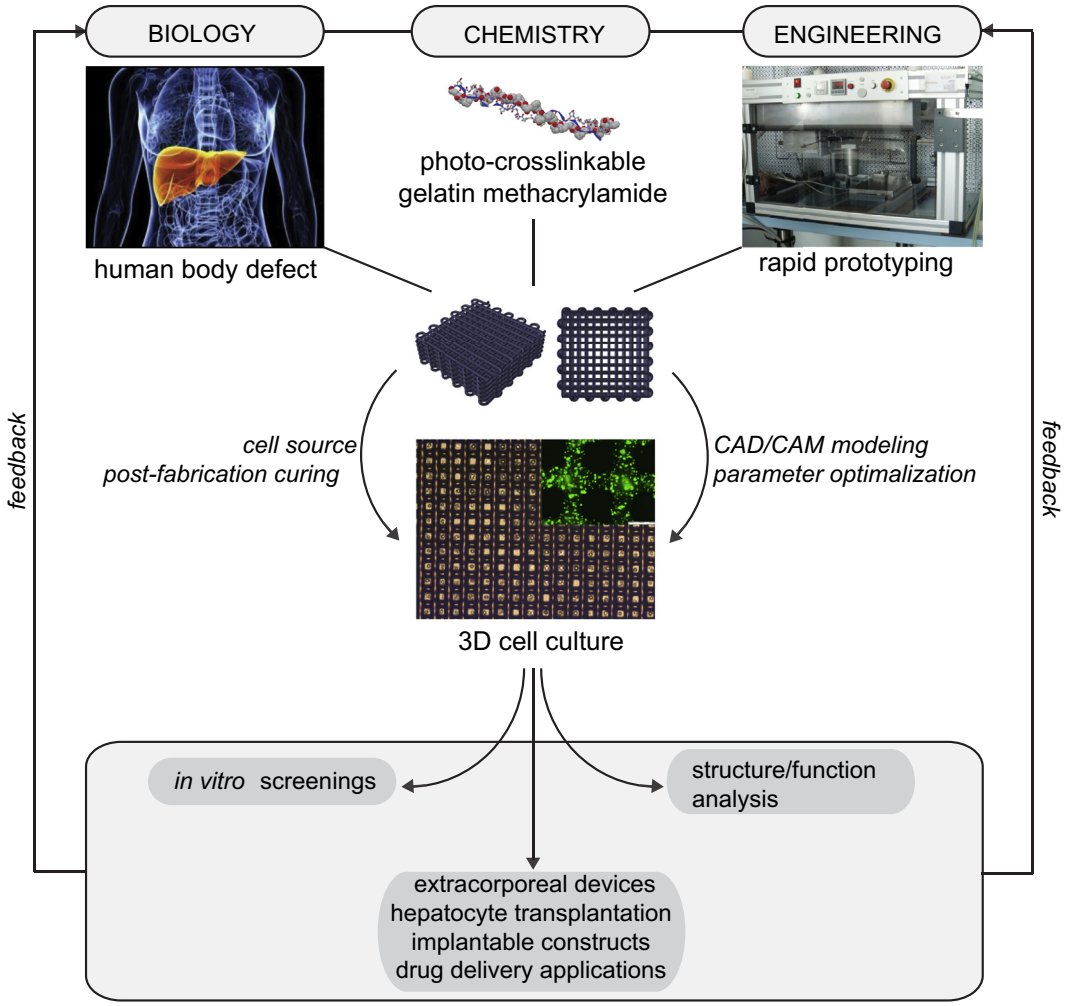


**Fig. 1.** Schematic illustration of multi- and interdisciplinary workflow related to the use of rapid prototyping in the field of tissue engineering.

developed an *in vitro* drug screening system via integration of a 3D cell-laden alginate hydrogel environment integrated within a microbioreactor.

Besides the previously mentioned advantages of 3D porous scaffolds, the introduction of pores has been reported to enhance both cell proliferation and albumin production of HepG2 cells in porous alginate hydrogels [24]. Earlier work demonstrated good *in vitro* cytocompatibility of photosensitive gelatin methacrylamides for the encapsulation of fibroblasts, myoblasts, chondrocytes endothelial cells, and cardiac cells [22,25–27]. Taking all this into account, the generation of a highly viable cell-laden gelatin scaffold with sufficient mechanical stability would be desirable.

The Bioplotter technology enabled researchers to generate hydrogel-based constructs. The present study aims to develop a 3D microenvironment applying post-processing photo-induced free-radical cross-linking of cell-laden gelatins. An evaluation of two photo-initiating systems is performed, introducing an alternative photo-initiator, and a parametric study on the printing of (cell-laden) gelatin hydrogels is presented.

## 2. Experimental part

### 2.1. Cell culture

HepG2 cells were maintained in a humidified 5% $CO_2$-containing atmosphere (37 °C) with cultivation medium consisting of DMEM, supplemented with 10 v% FBS, 50 U $ml^{-1}$ penicillin and 50 μg $ml^{-1}$ streptomycin, all provided by Life technologies.

### 2.2. Hydrogels

Bovine type B gelatin (approximate iso-electric point of 5 and Bloom strength of 257), isolated by alkaline treatment, was supplied by Rousselot (Ghent, Belgium). Photosensitive gelatin, gelatin methacrylamide, was synthesized (Scheme 1) as described in detail [28]. In short, a solution of gelatin type B in phosphate buffer at pH 7.8 was reacted with methacrylic anhydride (Sigma–Aldrich). The purified gelatin methacrylamide had a degree of substitution of 62%, as determined by $^1H$ NMR (Bruker AVANCE II 500 MHz) in deuterated water (Sigma–Aldrich) at 45 °C. Prior to use, the hydrogel building blocks were sterilized by ethylene oxide treatment (cold cycle, Maria Middelares hospital, Ghent, Belgium).

### 2.3. Photo-cross-linking presets

Two types of photo-initiators (PIs) were used in this paper: (i) 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure® 2959, I2959) was obtained from Ciba Specialty Chemicals (Groot-Bijgaarden, Belgium); (ii) 2,2′-Azobis[2-methyl-*N*-(2-hydroxyethyl)propionamide] (VA-086) photo-initiator was purchased from Wako Specialty Chemicals. I2959 or VA-086 stock solutions in PBS were filter sterilized and added to the culture medium or hydrogel building blocks to create respectively final concentrations of 2 mol% or 20 mol% (with respect to the amount of double bonds). These precursor solutions were thoroughly degassed for at least 10 min prior to loading with HepG2 cells. An LWUV lamp model VL-400L (Vilber Lourmat, Marne La Vallée, France) was used for (cell-laden) hydrogel sample curing (UV-A light, 365 nm, 4 mW $cm^{-2}$).

### 2.4. Rheological evaluation of gelatin methacrylamides

The effects of the applied PI on the *in situ* curing of gelatin methacrylamide precursor solutions (10 w/v %) were evaluated at 20 °C using a Physica MCR 350 (Anton Paar) with plate–plate geometry. For this, non-irradiated 2D slabs were