Title	Nanofibrillated cellulose hydrogel as a lyoprotectant in the freeze-drying of HepG2 3D spheroids
Keywords (up to 5)	Freeze-drying, lyoprotectant, cell spheroids, nanofibrillated cellulose, HepG2
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Abstract	Freeze-drying is a widely used method in pharmaceutical applications for dry preservation of heat-labile biological products. In the freeze-drying process the sample is first frozen and then dried in a pressure below the triple-point of water, when the water begins to sublimate resulting in a dry final product. The process is suitable for drying i.e. proteins, and usually excipients (lyoprotectants) are required. Until now, freeze-drying of complex human cells has not resulted in viable cells. Ability to freeze-dry and revive viable mammalian cell spheroids would be beneficial concerning tissue-engineering, drug research and other biomedical applications, because cells could be stored and transported in dry state. The aim of this project is to evaluate the lyoprotective features of nanofibrillated cellulose (NFC). It has been shown earlier that NFC promotes cells three-dimensional growth, spheroid formation and differentiation. When freeze-dried, NFC forms a highly porous aerogel structure and could serve as protective matrix for cells. Results show that cell spheroids freeze-dried in the NFC matrix had metabolic and esterase activity after rehydration. In addition, the morphology and the three-dimensional structure of the spheroids were preserved. However, freeze-dried and rehydrated cell spheroids were not able to proliferate or attach anymore and the cell-membrane was not intact. Cells freeze-dried without the NFC matrix showed no enzyme or metabolic activity. Current results show that NFC has some lyoprotective features, but because of the lack of cell attachment, other lyoprotectants should be studied with NFC and the freeze-drying cycle and rehydration process optimized to achieve cell attachment and higher cell viability for future applications.
References	1. Franks, F. Freeze-drying of bioproducts: putting principles into practice. *European Journal of Pharmaceutics and Biopharmaceutics* **45**, 221-229 (1998) 2. Bhattacharya, M. *et al*. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. *Journal of controlled release* **164**, 291-298 (2012).