Citation	Authors
	Carmen Piras, Susana Fernandez-Prieto, Wim M De Borggraeve
	2017 Nanocellulosic materials as bioinks for 3D bioprinting
	Journal Biomaterials Science Volume:5 Issue: Pages:1988-1992
Archived version	Manuscript before formatting by journal
Published version	http://dx.doi.org/10.1039/C7BM00510E
Journal homepage	http://pubs.rsc.org/en/journals/journalissues/bm
Author contact	wim.deborggraeve@kuleuven.be +32 (0)16 32 76 93
LIRIAS	https://lirias.kuleuven.be/handle/123456789/599323

(article begins on next page)

Biomaterials Science

MINIREVIEW

Nanocellulosic materials as bioinks for 3D bioprinting

Carmen C. Piras,^a Susana Fernández-Prieto^b and Wim M. De Borggraeve^aReceived 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

3D bioprinting is a new developing technology with lots of promise in tissue engineering and regenerative medicine. Being biocompatible, biodegradable, renewable and cost-effective, cellulosic nanomaterials have recently captured the attention of researchers due to their applicability as inks for 3D bioprinting. Although a number of cellulose-based bioinks have been reported, the potential of cellulose nanofibrils and nanocrystals has not been fully explored yet. This minireview aims at highlighting the use of nanocellulosic materials for 3D bioprinting as an emerging, promising, new research field.

Introduction

3D printing is an emerging technology that allows the layer-by-layer deposition of material in specifically designed 3D shapes. This high-precision manufacturing technique has recently gained prominent interest in tissue engineering, as it can be applied for the fabrication of 3D bioprinted scaffolds of living tissues.^{1,2} Advantages of this technology include high reproducibility, complex structure production, fine control of the process and up-scalability. Furthermore, it allows the direct embedment of cells inside the scaffold and the distribution of different cell types at desired locations. Numerous applications of 3D bioprinting have been reported in literature from bone and cartilage tissue engineered scaffolds,^{3,4} skin⁵ and prevascularised tissues fabrication,⁶ cardiac constructs,⁷ to hepatic tissue generation.⁸

As described by Suntornnond *et al.*, 3D bioprinting strategies can be mainly classified into three technologies: extrusion, inkjet and laser-assisted.² The extrusion is a versatile, widely employed method that can be applied to bioinks ranging from low to high viscosities. It allows the extrusion of the ink out of a nozzle by applying a mechanical or a pneumatic force. This technique offers the benefits of scalability and cost-efficiency, but it has lower resolution than other methods. The inkjet technology is usually applied to print cell-laden droplets at a high resolution. This system can be driven either by a piezoelectric transducer or by a heater, which creates an air bubble that expands allowing the ejection of the liquid through the nozzle. Advantages of this method include cost-efficiency, high resolution and speed. Nozzle clogging is, however, its major drawback. The laser-assisted method is more expensive

than the other two and has a limited scalability; nevertheless, it enables high-resolution patterning. In this technique, the laser power heats an absorbing layer, generating a pressure change that determines the ejection of the bioink.

A fundamental factor in 3D bioprinting is the selection of the material employed as a bioink, which has to be suitable with the technology used to dispense the material.⁹ In this regard, the mechanical properties of the bioink (*i.e.* viscosity and shear thinning behavior) should meet the requirements of the applied technology, ensuring biocompatibility and cell survival during the printing process.¹⁰ For these reasons, with their high water content and their similarity to the extracellular matrix, hydrogels are ideal candidates to be employed as bioinks. To better control the mechanical properties of the gels and to avoid the 3D printed shape to collapse due to low viscosity, multicomponent bioinks obtained by combining different polymers, or nanocomposites, obtained by encapsulating nanoparticles into the gel network, are often used. Examples of hydrogels currently employed in 3D bioprinting include synthetic compounds such as polyacrylamide derivatives and polyethylene glycols, and natural polymers such as gelatin, agarose, alginate, collagen, hyaluronic acid and chitosan.²

Being abundant, renewable, economical, biodegradable resources with high stiffness and biocompatibility, nanocellulosic materials are of great interest for the design and fabrication of bioinks.¹¹ This minireview will focus on recent developments in nanocellulosic materials for 3D bioprinting. Numerous reviews have already been published on biological applications of cellulose derivatives,¹²⁻¹⁵ however 3D bioprinting is a relatively new, emerging research area for this type of materials. This review will firstly discuss the current state of the art. The attention will then be shifted to areas of future development. The aim is to highlight the potential of cellulose derivatives for 3D bioprinting as a growing new field of research. We hope that this document

^a Molecular Design and Synthesis, Department of Chemistry, KU Leuven - University of Leuven, Celestijnenlaan 200F, box 2404, B-3001 Leuven, Belgium.

^b Procter and Gamble Brussels Innovation Center, Temselaan 100, 1853 Strombeek-Bever, Belgium.

will help to inform the rational design of novel cellulose based materials for future application in this area.

Current uses of nanocellulosic materials for 3D bioprinting

Cellulose is a linear polysaccharide composed of $\beta(1\rightarrow4)$ linked D-glucose units obtained from the biosynthesis of plants or bacteria. The term nanocellulose refers to processed materials or cellulosic extracts with one dimension in the nanometer range. Depending on the source and the preparation method, such isolated cellulosic materials can be categorised into three main groups: nanofibrillated cellulose, cellulose nanocrystals and bacterial cellulose.¹⁶ Cellulose nanofibrils can be extracted from raw materials by a combination of mechanical and chemical treatments.^{16,17} Mechanical shearing actions (e.g. high-pressure homogenization and grinding) are applied to obtain microfibrils from cellulosic fibers. This process can be facilitated by different chemical pretreatments such as acid hydrolysis, enzymatic reactions and TEMPO-mediated oxidation.¹⁸ Biological pre-treatments can also be applied to remove lignin and hemicellulose by enzymatic degradation in the presence of microorganisms, such as fungi.^{19,20} The resulting cellulose particles are long entangled fibrils containing both amorphous and crystalline regions. One of the features of these fibers is that they can form highly entangled networks with high viscosity values at concentrations below 1 wt.%. Cellulose nanocrystals are highly crystalline rod-like particles, which are mainly produced by acid hydrolysis.²¹ This process disrupts the hydrogen bonds and the amorphous fiber domains, leading to nanoparticles with a well-defined shape. Bacterial celluloses are microfibrils secreted in highly crystalline form by different bacterial types.²² Among these, *Komagataeibacter xylinus* (formerly known as *Gluconacetobacter xylinus* or *Acetobacter xylinum*) is the most widely investigated. The fibrils are obtained by separation from the bacterial bodies and the growth medium and their morphology depends on the applied culturing conditions.

As previously mentioned, nanocellulosic materials display many interesting properties for application in tissue engineering. Amongst others, these include cost-effectiveness, sustainability, environmental friendliness, biocompatibility, biodegradability and non-toxicity. Therefore, it does not surprise that cellulose nanofibers and nanocrystals have been employed as inks for 3D printing in tissue engineering, drug delivery and wound healing.

Tissue engineering

With their high viscosity and shear-thinning behavior, cellulose nanofibrils were proven to be ideal to create 3D printed freestanding, biocompatible constructs. One of the advantages of such materials is the possibility to control their mechanical properties to adapt to cell behavior and environment. The use of cellulose nanofibrils for 3D bioprinting of cellular

architectures was reported by Torres-Rendon *et al.*, who demonstrated that the resulting hydrogels could be processed into complex shapes, which could be used as a template for freestanding cell constructs.²³ The cellulose fiber suspensions were prepared by TEMPO-mediated oxidation under alkaline conditions, followed by homogenization using a pressure microfluidizer. The fibers had a content of carboxyl groups of 0.85 mmol/g, an average length of up to 3 μm and an average diameter of 2 nm. Hollow tubes based on nanofibrillated cellulose were obtained by an additive manufacturing technique, which allowed flexible adjustment of the structural dimensions. This process was performed in a custom-built extruder consisting of a copper housing and a polyether ether ketone extrusion nozzle. The designed geometries were cross-linked covalently or supramolecularly by glutaraldehyde or calcium chloride respectively or by a combination of the two. Interestingly, better mechanical properties (i.e. stiffness and elongation at break) were obtained when only calcium chloride was used. This was explained by flocculation and mesoscale aggregation processes inside the hydrogel walls due to the treatment with HCl during the glutaraldehyde cross-linking. Another decisive parameter to keep optimal rheological conditions of the printed constructs was that the samples were dried after extrusion into ethanol and then re-hydrated. This step was fundamental to maintain the physical integrity of the tubes. The obtained constructs could be employed for fibroblast cell culturing, followed by enzymatic degradation of the 3D scaffold. Material degradability is another advantageous property of cellulose nanofibers. Once the scaffold is printed, degradation should slowly occur to allow cells to produce their own extracellular matrix. The reported materials displayed several advantages including ease of preparation of the cellulose fibers, tunability of the mechanical properties of the tubes depending on the employed cross-linker and biocompatibility. However, the process to obtain them is quite complex compared to more traditional extrusion-based 3D printing techniques.

3D printed textured cellular architectures were also created by Studart and coworkers, who reported cellulose nanocrystal based inks for bioprinting.²⁴ The nanocrystals were extracted from wood pulp and dispersed in water using a planetary speed mixer. The resulting particles had an aspect ratio of 18, an average length of 120 nm and an average diameter of 6.5 nm. 3D printing was performed using ink-loaded syringes, which were mounted on the printer. The ink was released through different size micronozzles by a pneumatic system. Interestingly, the particles displayed shear-induced alignment along the printing direction, leading to an enhancement in the mechanical properties of the resulting 3D structures. This property could be exploited to design new materials with programmable reinforcement along the desired direction. The described 3D printing method is very simple and could be applied to prepare constructs with tailored rheology. Nevertheless, biological investigation was not performed in this study. Further research in this area is needed.

One of the main challenges for the hydrogels employed in bioprinting is to achieve shape fidelity while printing and

retaining the designed structure. This can be difficult to achieve if the selected bioink has a low viscosity. As previously mentioned, this problem can be overcome by using multicomponent bioinks, which contain two or more polymers to ensure optimal mechanical properties. Cellulose derivatives can be added in a bioink formulation as rheology modifiers to improve the ink printability and shape fidelity after printing. Sawkins *et al.* described a poly(lactic-co-glycolic acid) ink, which contained carboxymethyl cellulose as a viscosity modifier to optimize the rheology of the bioprinted constructs.²⁵ These were obtained by the extrusion method and had mechanical properties comparable to those of cancellous bone. Moreover, viable cells and active therapeutic proteins could be incorporated into the construct during the fabrication process thanks to the mild conditions employed during bioprinting. In this case, the cellulosic component not only allowed optimization of rheology parameters, but also offered a readily available platform for cell encapsulation by simple mixing.

Another example was reported by Henriksson and coworkers, who created a bioink for encapsulating adipocytes, based on nanocellulose and hyaluronic acid.²⁶ This ink was composed of sterilized nanofibrillated cellulose (diameter of about 20–30 nm) and hyaluronic acid in a 70:30 or 80:20 ratio. Before printing, the inks were mixed with the cell suspensions and then transferred into a cartridge, from which they were released by extrusion. The resulting 3D printed cell laden constructs could be used for *in vitro* biological studies on adipocytes. The cellulose nanofibrils displayed an isotropic distribution, which allowed the formation of a porous network with optimal conditions for cell culture. Furthermore, in such 3D printed culture systems, cells could accumulate more lipids than cells cultured using conventional 2D methods and displayed an increased gene expression of adipogenic marker genes PPAR γ and FABP4. Besides this, a better differentiation of mesenchymal stem cells into adipocytes was observed. Advantages of this type of ink included ease of preparation, as both the nanocellulose and the hyaluronic acid mixed together were commercially available, and compatibility with the cell encapsulation method.

Bioinks based on nanocellulose and hyaluronic acid were also studied by the Simmonson group, who investigated the applicability of nanocellulose/hyaluronic acid and nanocellulose/alginate composites for cartilage tissue engineering.²⁷ These inks were based on nanofibrillated cellulose obtained by mechanical refinement followed by enzymatic treatment, as reported by Paakko *et al.*²⁸ 3D printing was performed by extrusion and a subsequent treatment with calcium chloride to cross-link the obtained structures. The best results were achieved with 3D bioprinted cellulose/alginate constructs, which could support the growth and differentiation into chondrocytes of human-derived induced pluripotent stem cells. Although these composites were suitable for bioprinting, the method employed to obtain the cellulose fibers, which involves an enzymatic treatment, has the disadvantage of being rather expensive, especially for large-scale material preparation.

Studies on nanofibrillated cellulose/alginate bioinks were also conducted by Gatenholm and coworkers, who used this composite to 3D print several structures representing cartilage tissue with high shape fidelity and resolution.²⁹ The cellulose nanofibrils employed had an average width of 20 nm, an average charge density of 24 $\mu\text{eq/g}$ and a sugar composition of 95.3 % glucose, 1.7 % mannose and 0.7 % xylose. Also in this case, they were obtained by mechanical refinement and a subsequent enzymatic treatment.²⁸ Bioprinting was carried out by using a microvalve dispenser in the printer head based on electromagnetic jet technology for accurate jetting or contact dispensing. Compared to extrusion methods, this 3D printing technique has a better resolution. Four different bioinks were studied, which exhibited a proportional viscosity to the dry content of the ink rather than the ratio between the two components. This allowed controlling the cross-linking properties of alginate by simply changing the proportion of alginate to cellulose nanofibers, without influencing the mechanical properties and consequently the printability of the ink.

The studies conducted by this research group, were further expanded to develop a nanofibrillated cellulose/alginate based bioink for the regeneration of the auricular cartilage tissue.³⁰ This multicomponent hydrogel could support high density and homogeneous distribution of human nasal chondrocytes. The resulting bioink allowed the 3D printing of patient-tailored auricular constructs with controlled cell density and porosity, which showed remarkable shape and size stability. Furthermore, it allowed cell redifferentiation and the production of cartilage-specific extracellular matrix components. Also in this case, bioprinting was performed on a 3D printer equipped with two microvalve printheads based on electromagnetic jet technology and a thermoplastic extruder. A variant of this bioink was subsequently proposed by the Zenobi-Wong group, who introduced some sulfate functionalities into alginate to bind growth factors.³¹ The prepared alginate sulfate/nanofibrillated cellulose ink exhibited good printability, however the biological performance of the cells (*i.e.* cell growth, morphology and extracellular matrix deposition) was influenced by the nozzle geometry. 3D printing was undertaken by the extrusion technique using filtered compressed air through straight steel or conical needles having different diameters. The best preservation of the cell function was achieved with conical needles. This study demonstrated the importance of the applied printing conditions and their influence on cell behavior.

Drug delivery and wound healing

3D printing has also been employed for other biological applications such as drug delivery and wound healing.³² Pietrzak and coworkers, for example, reported a 3D printed flexible dose tablet system based on three methacrylic polymers and hydroxypropyl cellulose.³³ 3D printing was carried out by fused deposition modeling (FDM), an extrusion based technique in which polymer filaments are passed

through a high temperature nozzle, followed by fusion and subsequent solidification when cooling to room temperature. The obtained drug delivery system exhibited immediate and extended release of the model drug (theophylline) and the design could be finely adjusted to achieve an easy-to-swallow tablet design. This research showed high potential of 3D printing for individualized therapeutic treatments.

Further studies were conducted by Kempin *et al.*, who evaluated the 3D printability by FDM of different polymers (namely Eudragit_RS, polycaprolactone, poly(L-lactide) (PLLA) and ethyl cellulose) as drug loaded implants.³⁴ The drug release profile of the fluorescent dye quinine was investigated, which was highly dependent on the employed polymer and on the drug load. The results obtained demonstrated the applicability of cellulosic derivatives for 3D printed implants with controlled drug loading and delivery.

The uses of nanocellulose in wound healing have been previously described in a number of research articles and reviews.³⁵⁻³⁹ The group of Thomas explored the use of nanofibrillated cellulosic materials as bioinks for 3D printed porous structures as wound dressings.⁴⁰ The compounds were prepared by TEMPO oxidation and a combination of carboxymethylation and periodate oxidation. The nanofibrils obtained by periodate oxidation had smaller widths than 20 nm and shorter lengths than 200 nm, which determined a reduced viscosity of the formed hydrogels, leading to ideal mechanical properties to be employed as bioinks. 3D printing was performed by the extrusion method using a 3D bioplotter. The resulting layered structures were stabilized by ionic cross-linking after the addition of calcium chloride and subsequently freeze-dried. These 3D printed constructs, not only did not support bacterial growth, but also thanks to their porosity, showed the potential to incorporate and release antimicrobial drugs.

Further promising materials for wound healing were described by Leppiniemi *et al.*, who reported nanocellulose/alginate/glycerin composites suitable for 3D printing.⁴¹ The cellulose fibrils were obtained by TEMPO-oxidation from never-dried bleached hardwood kraft pulp, followed by treatment with a microfluidizer. 3D printing was performed by extrusion using a simplified pump system based on air pressure. The presence of cellulose fibrils, allowed avoiding the collapse of the printing paste; moreover, the presence of voids within the printed structure offered space for swelling in moist conditions.

Conclusions and future perspectives

3D bioprinting is a rapidly developing research area with huge potential. The studies reviewed showed the applicability of nanocellulosic derivatives in this field. Cellulose is a low-cost, readily available, biocompatible material, which represents a natural, sustainable resource, highly valuable for biological applications, *e.g.* tissue engineering, regenerative medicine, drug delivery and wound healing. Cellulose nanofibrils and nanocrystals have been widely employed in biology, however

their use as bioinks or in bioink formulations has not been yet fully explored. To our knowledge, the research that has been carried out so far mainly focuses on cellulose composites as bioinks for tissue engineering. A limited number of studies have also been reported on 3D printed drug delivery systems and wound dressings. Although the advantages of nanocellulose materials are well known in biology, their use in 3D bioprinting needs to be further investigated. Amongst others, one of the advantageous properties of nanocellulose that should be taken into consideration is rheology. This should be tuned according to the employed 3D printing technology or the specific tissue to be printed. The source of cellulose, the presence of impurities and the aspect ratio of the fibers (*i.e.* the length to width ratio) can have a remarkable impact on rheology. Modifications of the mechanical properties could be also achieved by simple variation of the dry matter concentration or by incorporation of nanoparticles into the gel network. The chemical functionalization of cellulose nanofibers and nanocrystals is another option. Nowadays, this can also be achieved in a sustainable way by adopting green technologies, such as the ball mill.^{42, 43} Furthermore, nanocellulosic materials can be used as rheology modifiers to improve the rheological performances of other polymers, ensuring to achieve optimal viscosity and shear-thinning behavior. For this reason, these materials are highly suitable to be employed together with other polymers in multicomponent bioink formulations.

Since this is a growing new field of research, we hope that this minireview will help to inspire the rational design of novel nanocellulose based bioinks for future applications in tissue engineering, regenerative medicine, drug delivery and wound healing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the FP7-PEOPLE-2013-IAPP EU Programme, for providing financial support to the research project ISSFLOW- FP7-PEOPLE-2013-IAPP-612330.

References

- 1 Y. S. Zhang, K. Yue, J. Aleman, K. Mollazadeh-Moghaddam, S. M. Bakht, J. Z. Yang, W. T. Jia, V. Dell'Erba, P. Assawes, S. R. Shin, M. R. Dokmeci, R. Oklu and A. Khademhosseini, *Ann Biomed Eng*, 2017, **45**, 148-163.
- 2 R. Suntornnond, J. An and C. K. Chua, *Macromol Mater Eng*, 2017, **302**, 1600266.
- 3 V. A. Seleznev and V. Y. Prinz, *Nanotechnology*, 2017, **28**, 1-9.
- 4 E. Axpe and M. L. Oyen, *Int J Mol Sci*, 2016, **17**, 1976.
- 5 D. Singh, D. Singh and S. S. Han, *Polymers-Basel*, 2016, **8**, 19.
- 6 X. X. Han, R. Bibb and R. Harris, *Biofabrication*, 2016, **8**, 025018.
- 7 K. M. Farooqi, O. Saeed, A. Zaidi, J. Sanz, J. C. Nielsen, D. T. Hsu and U. P. Jorde, *Jacc-Heart Fail*, 2016, **4**, 301-311.
- 8 Y. Kim, K. Kang, J. Jeong, S. S. Paik, J. S. Kim, S. A. Park, W. D. Kim, J. Park and D. Choi, *Ann Surg Treat Res*, 2017, **92**, 67-72.
- 9 D. Chimene, K. K. Lennox, R. R. Kaunas and A. K. Gaharwar, *Ann Biomed Eng*, 2016, **44**, 2090-2102.

- 10 K. Hölzl, S. M. Lin, L. Tytgat, S. Van Vlierberghe, L. X. Gu and A. Ovsianikov, *Biofabrication*, 2016, **8**, 032002.
- 11 C. Salas, T. Nypelo, C. Rodriguez-Abreu, C. Carrillo and O. J. Rojas, *Curr Opin Colloid In*, 2014, **19**, 383-396.
- 12 T. Huber, J. Mussig, O. Curnow, S. S. Pang, S. Bickerton and M. P. Staiger, *J Mater Sci*, 2012, **47**, 1171-1186.
- 13 C. Y. Chang and L. N. Zhang, *Carbohydr Polym*, 2011, **84**, 40-53.
- 14 R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem Soc Rev*, 2011, **40**, 3941-3994.
- 15 G. Siqueira, J. Bras and A. Dufresne, *Polymers-Basel*, 2010, **2**, 728-765.
- 16 Klemm, F. Kramer, S. Moritz, T. Lindstrom, M. Ankerfors, D. Gray and A. Dorris, *Angew Chem Int Edit*, 2011, **50**, 5438-5466.
- 17 A. Naderi, *Cellulose*, 2017, **24**, 1933-1945.
- 18 A. Dufresne, *Mater Today*, 2013, **16**, 220-227.
- 19 G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran and S. Ramakrishnan, *Enzyme Res*, 2011, **2011**, 787532.
- 20 C. Sanchez, *Biotechnol Adv*, 2009, **27**, 185-194.
- 21 M. Mariano, N. El Kissi and A. Dufresne, *J Polym Sci Pol Phys*, 2014, **52**, 791-806.
- 22 H. G. D. Barud, R. R. da Silva, H. D. Barud, A. Tercjak, J. Gutierrez, W. R. Lustri, O. B. de Oliveira and S. J. L. Ribeiro, *Carbohydr Polym*, 2016, **153**, 406-420.
- 23 J. G. Torres-Rendon, M. Kopf, D. Gehlen, A. Blaeser, H. Fischer, L. De Laporte and A. Walther, *Biomacromolecules*, 2016, **17**, 905-913.
- 24 G. Siqueira, D. Kokkinis, R. Libanori, M. K. Hausmann, A. S. Gladman, A. Neels, P. Tingaut, T. Zimmermann, J. A. Lewis and A. R. Studart, *Adv Funct Mater*, 2017, **27**, 1604619.
- 25 M. J. Sawkins, P. Mistry, B. N. Brown, K. M. Shakesheff, L. J. Bonassar and J. Yang, *Biofabrication*, 2015, **7**, 035004.
- 26 I. Henriksson, P. Gatenholm and D. A. Hagg, *Biofabrication*, 2017, **9**, 015022.
- 27 D. Nguyen, D. A. Hagg, A. Forsman, J. Ekholm, P. Nimkingratana, C. Brantsing, T. Kalogeropoulos, S. Zaunz, S. Concaro, M. Brittberg, A. Lindahl, P. Gatenholm, A. Enejder and S. Simonsson, *Sci Rep-Uk*, 2017, **7**, 1-10.
- 28 M. Paakko, M. Ankerfors, H. Kosonen, A. Nykanen, S. Ahola, M. Osterberg, J. Ruokolainen, J. Laine, P. T. Larsson, O. Ikkala and T. Lindstrom, *Biomacromolecules*, 2007, **8**, 1934-1941.
- 29 K. Markstedt, A. Mantas, I. Tournier, H. M. Avila, D. Hagg and P. Gatenholm, *Biomacromolecules*, 2015, **16**, 1489-1496.
- 30 S. Schwarz, H. M. Avila, N. Rotter and P. Gatenholm, *Tissue Eng Pt A*, 2015, **21**, S373-S373.
- 31 M. Muller, E. Ozturk, O. Arlov, P. Gatenholm and M. Zenobi-Wong, *Ann Biomed Eng*, 2017, **45**, 210-223.
- 32 G. Jonathan and A. Karim, *Int J Pharmaceut*, 2016, **499**, 376-394.
- 33 K. Pietrzak, A. Isreb and M. A. Alhnan, *Eur J Pharm Biopharm*, 2015, **96**, 380-387.
- 34 W. Kempin, C. Franz, L. C. Koster, F. Schneider, M. Bogdahn, W. Weitschies and A. Seidlitz, *Eur J Pharm Biopharm*, 2017, **115**, 84-93.
- 35 A. G. Dumanli, *Curr Med Chem*, 2017, **24**, 512-528.
- 36 T. Hakkarainen, R. Koivuniemi, M. Kosonen, C. Escobedo-Lucea, A. Sanz-Garcia, J. Vuola, J. Valtonen, P. Tammela, A. Makitie, K. Luukko, M. Yliperttula and H. Kavola, *J Control Release*, 2016, **244**, 292-301.
- 37 S. Napavichayanun, R. Yamdech and P. Aramwit, *Arch Dermatol Res*, 2016, **308**, 123-132.
- 38 M. Jorfi and E. J. Foster, *J Appl Polym Sci*, 2015, **132**, 41719.
- 39 J. Liu, G. Chinga-Carrasco, F. Cheng, W. Y. Xu, S. Willfor, K. Syverud and C. L. Xu, *Cellulose*, 2016, **23**, 3129-3143.
- 40 A. Rees, L. C. Powell, G. Chinga-Carrasco, D. T. Gethin, K. Syverud, K. E. Hill and D. W. Thomas, *Biomed Res Int*, 2015, **2015**, 925757. 10.1155/2015/925757.
- 41 J. Leppiniemi, P. Lahtinen, A. Pajanen, R. Mahlberg, S. Metsa-Kortelainen, T. Pinornaa, H. Pajari, I. Vikholm-Lundin, P. Pursula and V. P. Hytonen, *Acs Appl Mater Inter*, 2017, **9**, 21959-21970.
- 42 H. Y. Hu, H. X. Li, Y. J. Zhang, Y. M. Chen, Z. Q. Huang, A. M. Huang, Y. Q. Zhu, X. Z. Qin and B. F. Lin, *Rsc Adv*, 2015, **5**, 20656-20662.
- 43 S. Deng, R. Huang, M. Zhou, F. Chen and Q. Fu, *Carbohydr Polym*, 2016, **154**, 129-138.