Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biomedical Engineering

3D printing of nano-cellulosic biomaterials for medical applications

Sahar Sultan[a], Gilberto Siqueira[b], Tanja Zimmermann[b] and Aji P. Mathew[a,b]

Abstract

Nanoscaled versions of cellulose viz. cellulose nanofibers (CNF) or cellulose nanocrystals (CNC) isolated from natural resources are being used extensively since the past decade in the biomedical field e.g. for tissue engineering, implants, drug delivery systems, cardiovascular devices, and wound healing due to their remarkable mechanical, chemical and biocompatible properties. In the recent years, 3D printing of nanocellulose in combination with polymers is being studied as a viable route to future regenerative therapy. The printability of nanocellulose hydrogels owing to their shear thinning behavior and the possibility to support living cells allows 3D bioprinting using nanocellulose, a recent development which holds tremendous potential.

Addresses

[a] Department of Materials and Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden

[b] Applied Wood Materials Laboratory, Functional Cellulose Materials Group – Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland

Corresponding author: Mathew, Aji P, Department of Materials and Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden. (aji.mathew@mmk.su.se)

Current Opinion in Biomedical Engineering 2017, **2**:29–34

This review comes from a themed issue on **Additive Manufacturing**

Edited by **Seeram Ramakrishna, Carlijn V. C. Bouten and Roger Narayan**

Received 30 January 2017, accepted 2 June 2017

http://dx.doi.org/10.1016/j.cobme.2017.06.002

Keywords

Cellulose nanofibers, Cellulose nanocrystals, Biomedical, 3D printing, Bioink.

Introduction

3D printing also known as additive manufacturing (AM) or rapid prototyping (RP) follows a bottom up process and has been applied in medicine since the early 2000s to make dental implants and customized prostheses [1–3]. AM technologies such as direct ink writing (DIW), standard lithography, laser-based polymerization, or epitaxial assembly techniques can be employed in order to create 3D structured objects [4]. This review is focused on the DIW process, which is an extrusion-based technique that enables programmable assembly of three-dimensional periodic architectures.

An advantage over the other techniques is that a broad range of materials can be printed at the micrometer scale by DIW. The main challenge related to this DIW is assigned to the development of inks possessing optimum rheological behavior [5].

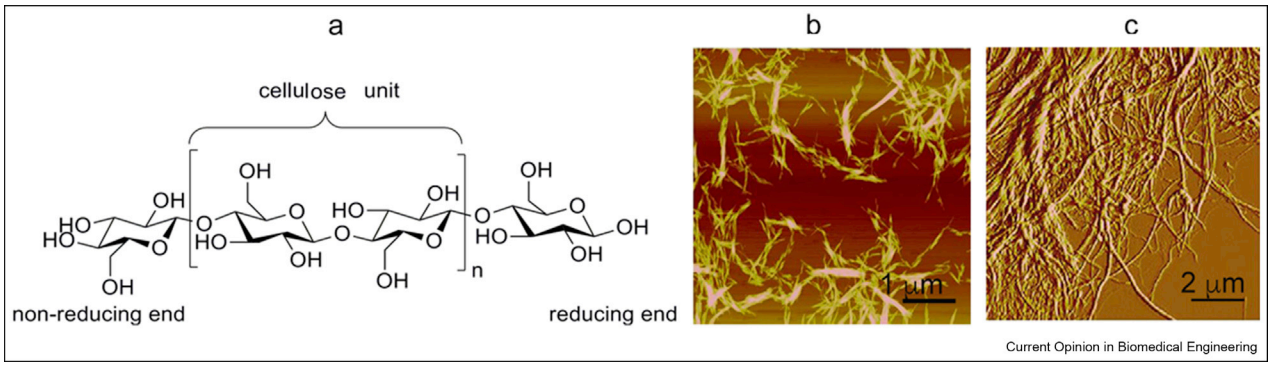
3D printing technology used in biomedical field, involves collecting accurate information of tissues and organs for designing the model, transferring the information into electrical signal to control the printer, and developing a printing process that maintain the cell viability during the fabrication process.

Cellulose is a linear carbohydrate polymer with long chains of β-(1 → 4)-linked D-anhydroglucopyranose moieties repeating units (Fig. 1a), it is made up of sugar monomers and is hence a polysaccharide. Nanocelluloses are isolated from diverse cellulose sources via a top down approach and known to have different nomenclature for the same type of nanocellulose as described below [6–8].

- Cellulose nanocrystals (CNC) (Fig. 1b), with other designations such as nanocrystalline cellulose, cellulose (nano)whiskers, rod-like cellulose microcrystals; which are the ordered regions in cellulose chains, isolated via acid or enzymatic hydrolysis and having diameters in the range of nanometers
- Cellulose nanofibers (CNF) (Fig. 1c), with the synonyms of nanofibrillated cellulose (NFC), microfibrillated cellulose (MFC), cellulose nanofibrils; comprising of the ordered and disordered regions of cellulose chains, usually isolated via mechanical disintegration, having diameters in nanoscale and lengths in micron range.

Abundance, wide availability, insolubility in water/solvents, biocompatibility etc are the reasons why nanocellulose is being considered in biomedical application. In the recent years, research activities in the field of "biomedical application of nanocellulose" has grown exponentially [6] and the use of 3D printing is one of the most recent and promising developments in this field. The specific reasons why nanocelluloses are used in 3D printing are that they can be utilized as rheological modifiers for the inks, ensuring the viscoelastic response required for filament printing; their specific mechanical

Fig. 1



a) Repeating unit of cellulose chemical structure and images of b) cellulose nanocrystals and c) cellulose nanofibers.

properties; their ability to support cells and the possibility to accurately control pore structure and shape in scaffolds and implants.

Compared to traditional fabrication approaches, the 3D printing technology allows fabrication of more complex structures by integrating layer by layer (bottom to top) slices of the designed and desired objects [9,10]. Moreover, it provides substantial liberty to fabricate new and untested geometric designs where, for instance, well-organized nanoscale building blocks can be assembled in a specific fashion to bridge structural length scales from nano-to-macro [11]. Hydrogel formation capability of nanocellulose at low concentrations (1–2 wt%) also has facilitated the design of inks for 3D printing.

The nomenclature in 3D printing of nanocelluloses for biomedical application is not standardized yet and different terms are being used to represent the techniques. In this review article, some terms are defined below for ease of comparison.

- Any biocompatible ink/hydrogel mixed with living cells is defined as **Bioink** and 3D printing of bioink is referred to as **3D Bioprinting**

In this review we aim to give a brief overview of factors to consider while 3D printing nanocellulosic inks and summarizes the recent research and commercial developments in 3D printed nanocellulosic systems for biomedical applications.

Factors affecting 3D printing

Majority of the current manufacturing processes using 3D printing is overwhelmingly based on single material printing, typically with a limited range of commercial and often proprietary resins compatible with commercial printers [10]. To overcome this issue and extend the application of 3D printing to biomedical products, researchers are adapting commercial printers and developing new inks based on naturally based polymers (including alginates, hyaluronic acid, gelatin, chitosan, collagen) [9] or synthetic polymers (polyethylene glycol; PEG) [12]. Materials that can be thermally crosslinked at body temperatures and/or that need short curing time, low photoinitiator concentrations, and low-intensity UV light to minimize possible adverse side effects on cells caused by creation of free radicals are generally considered attractive materials for 3D printing [13,14].

The selection of materials for 3D printing for biomedical applications and their performance are dependent on several characteristics; the most relevant are listed below:

- *Printability*: the importance of this parameter lies on the specific rheological behavior required for the inks used. For example, the ink has to be sufficiently fluid to be extruded through the micro nozzles under ambient conditions without demanding prohibitively high pressures (e.g. >4 bars). When shear ceases, the ink should exhibit enough elastic modulus (G'), typically higher than few kPa, and yield stress in the order of few 10^2 Pa, to maintain its filamentary shape thus preventing single filament deformation. One possible approach to meet the rheological requirements is the designing of the inks that display non-Newtonian viscoelastic response, generally evidenced by its high storage modulus (G') over the loss modulus (G'') at low shear stresses [5,15].
- *Biocompatibility*: the selected material should be compatible if it is aimed to coexist with endogenous tissue without causing undesirable effects in the host; [9].
- *Biodegradability*: since major part of developed scaffolds and constructs are not intended as permanent implants; [16].
- *Structural and mechanical properties*: the choice of materials should be based on the final mechanical properties required for the specific anatomical site into which it is intended to be implanted; [9,16].

The 3D bioprinting with cells or bioactive materials have all these challenges, but in addition requires rheological behavior that sustains cell viability during the printing process.

3D printed nanocellulose bioinks

Only few reports are available till date on nanocellulose 3D printing for biomedical application and Table 1 below summarizes the research reports on the topic of 3D printed biomedical materials.

Gatenholm and coworkers, Sweden, developed 3D printed nanocellulose scaffolds for biomedical applications. Markstedt et al. applied a bioink of CNF and alginate formulated for the 3D bioprinting of living soft tissue with cells [17]. $CaCl_2$ was used as the cross-linking agent for the alginate matrix. CNF provided shear thinning (10^5—10^{-1} Pa s) with high shape fidelity and good printing resolution (3.5 mm—0.5 mm). After cross-linking, the properties of alginate appeared to be dominant. To investigate any potential harmful effects of the ink on cell viability, in-situ and ex-situ cytotoxicity tests were performed. The results showed that the tested bioink is biocompatible and a suitable material for cell culture growth. However, the preparation and mixing caused first a decrease in viability but then a significant increase in cell viability was detected in the printed constructs after 7 days of 3D culture (85.7 ± 1.9%) compared to day 1 (72.8 ± 6.0%). The study concludes that a bioink composed of CNF and alginate is a suitable hydrogel for 3D bioprinting with living cells for growth of cartilage tissue.

As cellulose based bioinks gained huge popularity, some products are being commercialized, eg. CELLINK®, a biocompatible ink from CELLINK AB (Sweden) which has the potential to be used as bioink. CELLINK® is prepared in aseptic conditions and contains 2% (w/w) of plant-derived nanofibrillated cellulose (CNF) and 0.5% (w/w) of sterile sodium alginate. Gatenholm and coworkers evaluated the biological functionality of CELLINK® in a 28-days 3D culture and its potential use for auricular cartilage tissue engineering; aiming to provide an alternative and effective treatment to serious acquired or congenital auricular defects [18]. Here, also the matrix phase alginate was crosslinked by $CaCl_2$ solution. Human nasal chondrocytes (hNC) were mixed with the ink to obtain a bioink having a final concentration of 20×10^6 cells mL^{-1} and printed into auricular constructs with open porosity; see Fig. 2. Cell viability was examined throughout the bioprinting process: before embedding the hNCs in the ink, before embedding and crosslinking, and after embedding, bioprinting and crosslinking. The mean cell viability after embedding and crosslinking processes (68.5—76.9%) was found to be significantly lower than before embedding the hNCs (96.5 ± 2.1%). Furthermore, there was no significant difference between the after embedding and after bioprinting (70.9—77.2%) conditions, indicating that the bioprinting process had no significant influence on cell vitality. hNCs proliferated and underwent chondrogenesis by 3D culture in bioink, which resulted in neo-synthesis and accumulation of cartilage-specific extracellular matrix around the cells. Based on these results it was concluded that this bioink is promising for auricular cartilage tissue engineering and many other biomedical applications.

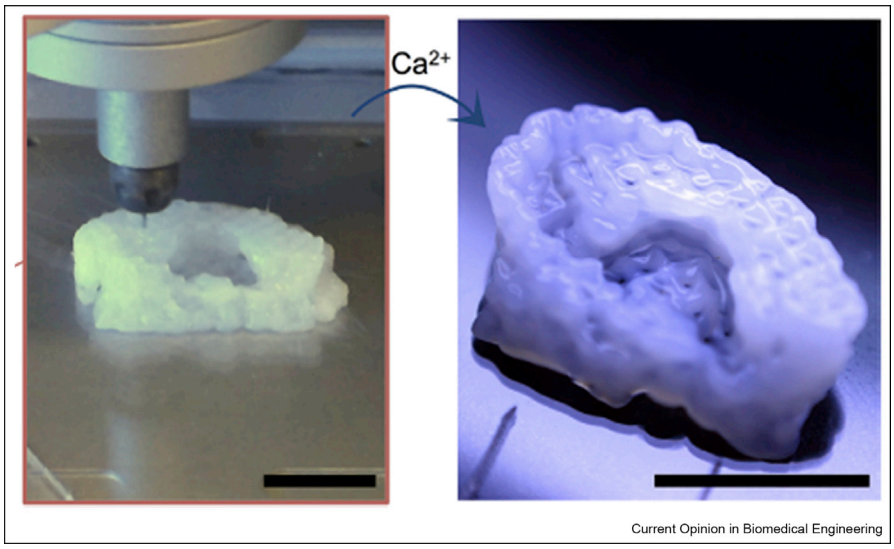
A mitogenic hydrogel system based on CNF was reported by Müller et al. in which alginate sulfate was used as a matrix [19]. To convert alginate sulfate into a printable bioink, it was combined with nanocellulose, which provided very good printability. Cell spreading properties were maintained with the optimal extrusion pressure (6—74 kPa) and shear stress for cell viability (<160 Pa). Despite the presence of nanocellulose, the mitogenic effect of alginate sulfate— nanocellulose and showed superior cell proliferation when compared with alginate—nanocellulose. Müller et al. describes that lower extrusion

Table 1

Summary of the reports on nanocellulose based biomedical materials.

Raw material	%/CNF/CNC	Matrix/crosslinker	Viscosity (Pa s)	Equipment	Application	Reference
Wood cellulose	CNF 2.5%	Alginate/$CaCl_2$	~10^5 @ 0.001 (s^{-1})	3D Discovery regenHU (Switzerland)	Cartilage tissue growth	[17]
Plant cellulose	CELLINK® CNF 2%	Alginate/$CaCl_2$	na	3D Discovery regenHU (Switzerland)	Auricular cartilage regeneration	[18]
Plant cellulose	CELLINK® CNF 1.9%	sulfated alginate/$CaCl_2$	~10^4 @ 0.01 (s^{-1})	BioFactory, regenHU, Switzerland)	Cartilage bioprinting applications	[19]
Plant cellulose	Methylcellulose	Alginate/$CaCl_2$	~10^6 @ zero shear	BioScaffolder 2.1-GeSiM (Germany)	Tissue engineering	[20]
Pinus radiata bleached kraft pulp	Tempo CNF, Carboxymethyl and periodate oxidized CNF	Tempo CNF/$CaCl_2$	~10^1 @ 10 (s^{-1})	3D Bioplotter (EnvisionTEC GmbH)	Wound dressing	[21]

Fig. 2



3D bioprinting process of chondrocyte-laden bioink and crosslinking with $CaCl_2$. Reproduced with permission from Ref. [18].

pressures and shear stresses, given by conical needles with wide diameter, provide the best preservation of cell function when printing chondrocytes in a CNF based bioink [19].

Schütz et al. used functionalized cellulose for the fabrication of centimeter-scaled tissue engineering constructs with tailored architecture by 3D bioplotting of an optimized alginate/methylcellulose (Alg/MC) hydrogel [20]. The addition of methylcellulose to a low concentrated alginate solution strongly improved the printability of the hydrogel material, enabling 3D bioplotting of constructs with tailored architecture and of high shape fidelity. The matrix of the hydrogel was crosslinked with $CaCl_2$. The Alg/MC material is also suitable to produce a cell-laden matrix: no cytotoxic effect of MC was detected and, after 21 days of cultivation within the Alg/MC matrix, high cell viability was found. Additionally, embedded hMSC were proven to maintain their differentiation into the adipogenic lineage as a model for soft tissue engineering that can be transferred to other cell types to follow the concept of organ printing. This 3D construct showed potential for generating tissue substitutes for the regenerative therapy of a number of different tissue types including fat and cartilage, and even for the regeneration of defects at tissue interfaces.

Rees et al. used homogenized cellulose nanofibers obtained after pretreated with a combination of carboxymethylation and periodate oxidation defined C-Periodate, as an ink for 3D Printing where TEMPO oxidized cellulose nanofibers was used as substrate [21]. The crosslinker, $CaCl_2$, stabilized the structures by forming ionic links between the carboxyl groups ($COO-$) and the divalent cations (Ca^{2+}). The combination of carboxymethylation and periodate oxidation led to a homogeneous material with short nanofibrils,

Table 2

News/press releases across the world about cellulose.

Date and place	Press release/news topic	Link
2016-01-25 USA	4D-printed structure changes shape when placed in water	http://news.harvard.edu/gazette/story/2016/01/4d-printed-structure-changes-shape-when-placed-in-water/
2015-05-17 Sweden	Cellulose from wood can be printed in 3D	http://www.chalmers.se/en/departments/chem/news/Pages/Cellulose-from-wood-can-be-printed-in-3D.aspx
2016-07-10 Sweden	Innventia starts project on 3D-printed prostheses based on forest raw materials	http://www.innventia.com/en/About-us/News1/Innventia-starts-project-on-3D-printed-prostheses-based-on-forest-raw-materials/
2016-07-07 USA	American process Inc. announces joint development agreement for 3D Printing of living cartilage tissue using nanocellulose for facial reconstruction	http://www.prweb.com/releases/2016/07/prweb13536943.htm

having widths <20 nm and lengths <200 nm. The small dimensions of the nanofibrils reduced the viscosity of the nanocellulose (10−0.1 Pa s), thus yielding a material with good rheological properties for use as a 3D printable ink. These nanocellulose 3D structures form tracks with an open porosity and the potential to carry and release antimicrobial components. It was demonstrated that the nanocellulose assessed in this study did not support bacterial growth and therefore having a distinct advantage for wound dressing applications.

Siqueira et al. [22] designed viscoelastic CNC-based inks for pattering three-dimensional objects. The materials were produced by direct ink writing and the shear induced alignment of CNCs during printing enabled the fabrication of textured composites with enhanced stiffness along the printing direction. The developed approach opens new opportunities for the development of bioinks for the biomedical fields.

Press releases on nanocellulose 3D printing

People across the globe are showing interest for nanocellulose based innovations in the context of 3D printing and press releases on this topic are summarized in Table 2.

Conclusion

The use of nanocellulose in 3D printing is a recent development and the gel forming properties of nanocellulose at low concentration and the shear thinning behavior combined with the biocompatibility, nontoxicity and good mechanical properties are all considered favorable for 3D printing of nanocellulosic implants, wound dressing materials, tissue engineering materials etc. where accurate control of pore structure and shape/geometries is beneficial. Nanocellulose scaffold processing based on 3D printing involves strategies either to 1) print using an ink laden with cells or 2) print the scaffold with biocompatible materials and subsequent cell seeding were found to be successful.

Although the 3D printing and/or bioprinting field faces challenges in terms of specific technical, material and cellular aspects of the process, the 3D printing technology is considered as one of the most advanced enabling technologies in tissue engineering and regenerative medicine field. It may be noted that majority of the reports are based on fibrillated cellulose (CNF) and inks with low concentrations (<5 wt%) of nanocellulose. Here, the limiting factor is the considerable viscosity increase induced by the nanofibrilated cellulose already at low concentrations. Use of high concentration inks are expected in the near future which will this technology to achieve materials with new mechanical, structural and functional performance.

Acknowledgments

AP Mathew acknowledges the financial support from Knut and Wallenberg Foundation through Wallenberg Wood Science Centre.

References

1. Gross Bethany C, Erkal Jayda L, Lockwood Sarah Y, Chen Chengpeng, Spence Dana M: **Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences**. *Anal Chem* 2014, **86**(7):3240.
2. Matsumoto Kunihito, Ishiduka Toru, Yamada Hisaya, Yonehara Yoshiyuki, Arai Yoshinori: **Clinical use of three-dimensional models of the temporomandibular joint established by rapid prototyping based on cone-beam computed tomography imaging data**. *Oral Radiol* 2014, **30**:98.
3. Gu Qi, Hao Jie, Lu Cangjie, Wang Liu, Wallace Gordon G, Zhou Qi: **Three-dimensional bio-printing**. *Sci China Life Sci* 2015, **58**:411.
4. Lewis J: **Direct ink writing of 3D functional materials**. *Adv Funct Mater* 2006, **16**:2193–2204.
5. Studart AR: **Additive manufacturing of biologically-inspired materials**. *Chem Soc Rev* 2016, **45**:359.
6. Klemm D, Heublein B, Fink H, Bohn A: **Cellulose: fascinating biopolymer and sustainable raw material**. *Angew Chem Int Ed* 2005, **44**:3358–3393.
7. Habibi Y, Lucia LA, Rojas OJ: **Cellulose nanocrystals: chemistry, self-assembly, and applications**. *Chem Rev* 2010, **110**:3479–3500.
8. Siró I, Plackett D: **Microfibrillated cellulose and new nanocomposite materials: a review**. *Cellulose* 2010, **17**:459–494.
9. Murphy Sean V, Atala Anthony: **3D bioprinting of tissues and organs**. *Nat Biotech* 2014, **32**:773.
10. Kalsoom U, Nesterenko PN, Paull B: **Recent developments in 3D printable composite materials**. *RSC Adv* 2016, **6**:60355.
11. Torres-Rendon JG, Femmer T, De Laporte L, Tigges T, Rahimi K, Gremse F, Zafarnia S, Lederle W, Ifuku S, Wessling M, *et al.*: **Bioactive gyroid scaffolds formed by sacrificial templating of nanocellulose and nanochitin hydrogels as instructive platforms for biomimetic tissue engineering**. *Adv Mater* 2015, **27**:2989–2995.
12. Guiding Gao XC: **Three-dimensional bioprinting in tissue engineering and regenerative medicine**. *Biotechnol Lett* 2016, **38**(2):203.
13. Censi R, van Putten S, Vermonden T, di Martino P, van Nostrum CF, Harmsen MC, Bank RA, Hennink WE: **The tissue response to photopolymerized PEG-p(HPMAm-lactate)-based hydrogels**. *J Biomed Mater Res Part A* 2011, **97A**:219–229.
14. Vermonden Tina, Fedorovich Natalja E, van Geemen Daphne, Alblas Jacqueline, van Nostrum Cornelus F, Dhert Wouter JA, Hennink Wim E: **Photopolymerized thermosensitive hydrogels: synthesis, degradation, and cytocompatibility**. *Biomacromolecules* 2008, **9**:919.
15. Compton BG, Lewis JA: **3D-Printing of lightweight cellular composites**. *Adv Mater* 2014, **26**:5930–5935.
16. O'Brein FJ: **Biomaterials & scaffolds for tissue engineering**. *Mater Today* 2011, **14**:88.
17. Markstedt Kajsa, Mantas Athanasios, Tournier Ivan, Ávila Héctor Martínez, Hägg Daniel, Gatenholm Paul: **3D bioprinting human chondrocytes with nanocellulose–alginate bioink for cartilage tissue engineering applications**. *Biomacromolecules* 2015, **16**:1489–1496.
18. Martínez Ávila H, Schwarz S, Rotter N, Gatenholm P: **3D bioprinting of human chondrocyte-laden nanocellulose hydrogels for patient-specific auricular cartilage regeneration**. *Bioprinting* 2016, **1–2**:22–35.
19. Müller Michael, Öztürk Ece, Arlov Øystein, Gatenholm Paul, Zenobi-Wong Marcy: **Alginate sulfate–nanocellulose bioinks**

for cartilage bioprinting applications. *Ann Biomed Eng* 2016: 1–14. http://dx.doi.org/10.1007/s10439-016-1704-5.

20. Schütz K, Placht A, Paul B, Brüggemeier S, Gelinsky M, Lode A: **Three-dimensional plotting of a cell-laden alginate/methylcellulose blend: towards biofabrication of tissue engineering constructs with clinically relevant dimensions**. *J Tissue Eng Regen Med* 2015. http://dx.doi.org/10.1002/term.2058.

21. Rees Adam, Powell Lydia C, Chinga-Carrasco Gary, Gethin David T, Syverud Kristin, Hill Katja E, Thoma David W: **3D bioprinting of carboxymethylated-periodate oxidized nanocellulose constructs for wound dressing applications**. *BioMed Res Int* 2015:925757–925765. http://dx.doi.org/10.1155/2015/925757.

22. Siqueira Gilberto, Kokkinis Dimitri, Libanori Rafael, Hausmann Michael K, Sydney Gladman Amelia, Neels Antonia, Tingaut Philippe, Zimmermann Tanja, Lewis Jennifer A, Studart André R: **Cellulose nanocrystal inks for 3D printing of textured cellular architectures**. *Adv Funct Mater* 2017:1604619.