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Brief Communication

# Three-dimensional printing with polylactic acid (PLA) thermoplastic offers new opportunities for cryobiology

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ABSTRACT

Development of devices through design, prototyping, testing, and fabrication is especially necessary for enhancement of research and eventual application in cryobiology. The advent of 3-dimensional printing offers unique opportunities for this process, given that the materials involved are suitable for use in cryogenic temperatures. We report herein that 3-D printing with polylactic acid (PLA) thermoplastic is ideally suited for cryobiology device development. Devices that are designed and standardized in open-source fashion can be electronically distributed and created locally on increasingly affordable 3-D printers, and can accelerate cryobiology findings and improve reproducibility of results.



Specialized devices are essential for collecting, handling, processing, and storing of samples for research and application. Such devices are intrinsic to cryobiology research, but pose the inherent problems of dealing with cryogenic temperatures [3]. As such, these devices are often expensive and designed for highly specialized purposes (such as freezing of human embryos [6]) which reduce their utility for adaptation for other applications (such as vitrification of fish sperm [2]). The paucity of existing devices also constrains the starting points for development of new devices, and accordingly cryobiological devices often have to be crafted from simple or readily available materials such as French straws [11]. In response to these problems, we have investigated the use of polylactic acid (PLA), a renewable biopolymer, as a material for prototyping and fabricating devices by 3-D printing suitable for cryogenic applications. The thermal properties of PLA allow devices created from this material to remain pliable and ductile at room and cryogenic temperatures and creates broad-ranging opportunities for cryobiology research and application.

Three-dimensional printing is becoming increasingly available and is being exploited in various applications. There are many different existing mechanisms, but fused deposition modeling (FDM) is one of the cheapest and easiest, and has advantages over other 3-D printing methods such as stereolithography (SLA) which uses a laser to cure liquid photopolymer resins. There are several FDM thermoplastics available, but in general they are heated and extruded through a precision-controlled nozzle that deposits a thin stream of the molten material in layers along a programmed X-Y-Z coordinate system. The resulting objects are reproducible, and based on the algorithms employed, can be printed as solid structures or with varying degrees of infill (internal honeycombing). Compared to SLA and other printing methods, FDM-printed objects are ideal for cryogenic applications as they are often porous, with low density, low thermal mass, and a high strength-to-weight ratio. The FDM thermoplastics involved are available in the form of filament of different thicknesses (e.g., 1.75–3 mm) wrapped around a spool or reel, and typically sold by mass (e.g., 1 kg) or printing time (e.g., 18–24 h). As such, these thermoplastics are cheap ($US 25–50 depending on quality and characteristics) and are available in multiple colors (including fluorescent) with physical properties such as being dissolvable by solvents to assist in printing complex (often overhanging) structures [12]. Over the past 3 years we have gained considerable experience in 3-D printing with the two most common thermoplastics, PLA and acrylonitrile butadiene styrene (ABS), and have found PLA to be particularly useful for cryogenic

*Abbreviations:* PLA, polylactic acid; ABS, acrylonitrile butadiene styrene; FDM, fused deposition modeling; SLA, stereolithography.

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applications.

In general, as most plastics are cooled towards cryogenic temperatures, they become harder, stiffer, and more brittle to mechanical loads, as evidenced by the typical shattering of plastics following cooling with LN2 [4]. Except for a few polymers specifically formulated for cryogenic applications, plastics exhibit a decrease in tensile elongation, the degree of stretch prior to breaking, as temperature decreases. Also observed as plastics are cooled is an increase in the elastic modulus, the ratio of applied stress to elongation, often described as stiffness. Depending on the formulation, plastics will undergo a ductile-to-brittle transition at some point below 0C, and thus will fail by snapping rather than deforming when compared to room temperature behavior. In our experience, objects 3-D printed with PLA can undergo considerable elongation prior to breaking (similar to room temperature) with spring-like designs remaining flexible at LN2 temperatures (Fig. 1). Even when cooled, these objects act more like springs with elastic behavior rather than undergoing irreversible plastic deformation, making them useful in cryogenic environments.

Other thermal properties of PLA also make it suitable for cryogenic applications. In our experience 3-D printed PLA objects do not appreciably warp when cooled and also can be safely handled immediately after removal from LN2. Most amorphous plastics have much lower thermal conductivity than metals, and thus do not efficiently transfer heat between temperature extremes. Even though plastics have higher specific heats than metals, and thus can store more heat per unit mass, because most 3-D printed objects have relatively low density (due to the honeycomb infill of structures that produces porosity) they cannot effectively withdraw and store heat, thus reducing risk of freeze injuries to personnel. Also, because plastics typically have much larger thermal expansion coefficients than metals, they shrink when chilled and present challenges in cryogenic designs. In contrast, the thermomechanical behavior of PLA observed by us showed little geometric distortion compared to other thermoplastics that can warp when printed onto a cool build plate or when cryogenically cooled. Because PLA has a lower glass transition (Tg) temperature than ABS and many other plastics, it can be extruded at cooler temperatures, and when deposited, cools in uniform fashion, thus preventing buildup of internal stresses in the object that could cause distortion when exposed to cryogenic conditions [8].

It is important to note that the aforementioned mechanical and thermal properties of 3-D printed objects and their filament materials are governed by polymer formulation as well as the printing process. At present, many of the exact formulations of filaments, as well as their material properties, are held as proprietary information by manufacturers. There are few scientific reports of materials testing on PLA filament but testing is increasing along with the popularity and utility of this manufacturing field. Specific designs and printing processes can also affect these properties, and printing parameters such as infill pattern and orientation, 3-D geometry slicing, shell and layer thicknesses are all worthy of consideration [10].

**Fig. 1.** A spring-like object (a wrist bracelet) 3-D printed with polylactic acid (PLA) thermoplastic filament held unstretched (left panel) and stretched (right) while submerged in liquid nitrogen. The object remained flexible at cryogenic temperatures and returned to its original state without damage.

At present we have recognized a number of potential uses of 3-D printed cryogenic devices, including racks and devices used for freezing, racks for sample storage, and devices used for sorting and transferring of frozen samples. Traditionally, plastic objects have been created by machining a prototype from a block of material that is produced in volume by injection molding, which is a slow, laborious and expensive process. With 3-D printing, one can rapidly prototype unlimited variations and fine tune these designs inexpensively. If the capabilities of local printers do not meet the needs of the design in terms of print size, resolution, or speed, there are online printing services (e.g., 3DHubs.com, MakeXYZ.com) where one can electronically submit designs to companies with more advanced printers, and have printed objects shipped to them.

The 3-D printing process begins with an idea of the object geometry defined in a computer-aided design (CAD) program, such as traditional engineering packages (e.g., AutoCAD, Autodesk Inventor, SolidWorks, Pro-Engineer) or newer freeware options [5] which use 3-D rendering to visualize the object and examine its features. This design file is processed through a Slicer program which uses an algorithm to convert it into layers for printing with consideration of specific printer attributes (layer thickness, extrusion speed and temperature) as well as identifying parameters such as printing orientation and selection of infill geometry. Once printed, the device can be tested, and if necessary, modified in the CAD program or Slicer algorithm and be iteratively optimized until it performs suitably. There is a sizeable and growing community of 3-D printing users and developers connected through the internet, and a wealth of designs and files already available as starting points. Designs that have broad applicability can be distributed as open-source files and be printed by others, thereby strengthening these communities through standardization and reproducibility of results, a consideration that is now receiving increasing attention from agencies such as the US National Institutes of Health [1].

As stated above, a number of research areas in cryobiology have been hindered due to the lack of specialized and suitable devices. It is clear that 3-D printing can offer solutions to address these problems. Three-dimensional printing has offered tremendous opportunities to other fields such as medical prosthetics where designs requiring custom fit can be created from scanned patient body geometries, inexpensively printed, and fitted to the end user [7]. Similarly for cryogenic applications, 3-D printing can accelerate progress in development of custom devices for handling, sorting, freezing, and storage of cryobiological samples as has been seen for technologies such as microfabrication [9]. 3-D printers continue to advance in capability and decrease in cost, and are now affordable for individual laboratories (~$2500) and should receive greater attention in cryobiology research and application. Use of the technology does not require specialized software or instrumentation expertise, as commercial markets are targeting the lives of everyday users, printing everything from toys to housewares, and therefore merits greater attention for cryobiological application.

## Conflict of interest

The authors do not have any conflict of interest.

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## References

[1] F.S. Collins, L.A. Tabak, Policy: NIH plans to enhance reproducibility, Nature 505 (2014) 612–613.
[2] R. Cuevas-Uribe, H. Yang, J. Daly, M.G. Savage, R.B. Walter, T.R. Tiersch, Production of F(1) offspring with vitrified sperm from a live-bearing fish, the green swordtail *Xiphophorus hellerii*, Zebrafish 8 (2011) 167–179.
[3] K.R. Diller, Engineering-based contributions in cryobiology, Cryobiology 34 (1997) 304–314.
[4] S.-Y. Fu, Cryogenic Properties of Polymer Materials, Polymers at Cryogenic Temperatures, Springer, 2013, pp. 9–39.
[5] S. Junk, C. Kuen, Review of Open Source and Freeware CAD Systems for Use with 3D-Printing, vol. 50, Procedia CIRP: College International pour la Recherche en Productique, 2016, pp. 430–435.
[6] M. Kuwayama, Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method, Theriogenology 67 (2007) 73–80.
[7] C. Lunsford, G. Grindle, B. Salatin, B.E. Dicianno, Innovations With 3-Dimensional Printing in Physical Medicine and Rehabilitation: A Review of the Literature, Physical Medicine and Rehabilitation, (2016) Epub ahead of print; DOI:http://dx.doi.org/10.1016/j.pmrj.2016.07.003.
[8] A. Salem Bala, S. bin Wahab, Elements and materials improve the FDM products: a review, Adv. Eng. Forum, Trans. Tech. Publ. (2016) 33–51.
[9] T. Scherr, G.L. Knapp, A. Guitreau, D.S. Park, T. Tiersch, K. Nandakumar, W.T. Monroe, Microfluidics and numerical simulation as methods for standardization of zebrafish sperm cell activation, Biomed. Microdevices 17 (2015) 65.
[10] B.M. Tymrak, M. Kreiger, J.M. Pearce, Mechanical properties of components fabricated with open-source 3-D printers under realistic environmental conditions, Mater. Des. 58 (2014) 242–246.
[11] G. Vajta, P. Holm, M. Kuwayama, P.J. Booth, H. Jacobsen, T. Greve, H. Callesen, Open Pulled Straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos, Mol. Reprod. Dev. 51 (1998) 53–58.
[12] http://3dprint.com, accessed 8.16.2016.