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Controlling implant shape remotely: 4D printed materials for improved health and medical applications

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Abstract

The objective of this study was to develop 4D printed materials for biomedical applications. 4D printed materials are materials which can change shape over time and on-demand upon exposure to external stimuli (i.e., heat, humidity, light, etc.). Here, a simple cytocompatible material is presented which can change shape in a controllable way when heated to body temperatures (37 °C), retain that shape at room temperature, and then change into its original shape when heated again to 37 °C. As an in vitro proof of concept for the promise of 4D printed materials, this present in vitro study examined stem cell delivery to treat neurological diseases (such as Parkinson's disease). Specifically, this material was seeded with model neurons (PC-12 cells), underwent a shape change from a flat shape suitable for cell culture to a tubular shape suitable for cell delivery and back to a flat shape showing no change in PC-12 cell number or neurite extensions per neuron, thus, demonstrating its suitability as a novel stem cell delivery device. This is in contrast to conventional cell delivery techniques which can significantly decrease cell viability by up to 70% due to the use of harsh enzymes (such as trypsin) needed to lift cells from flat tissue culture polystyrene to an injectable form. Although requiring more study, such 4D printed materials can also be used to straighten the spine of scoliosis patients, close aging weakened sphincters to treat acid reflux, and for the on-demand increase in pressure to regenerate intervertebral disk tissue for spinal applications, among many other applications.

Keywords: 3D printing, 4D printing, cell delivery, biomedical applications, neurological diseases, injectable materials, scoliosis, temperature-responsive materials

Introduction

Since their first development to today, medical devices (such as catheters, hip implants, pacemakers, or any conventional medical device) have been and continue to be static. They do not change shape, geometry, or architecture with time before or after implantation. While many would attribute the development of static implants necessary for quicker regulatory approval, there are numerous medical applications where developing materials that can change shape with time are beneficial. 4D printing is defined as the use of materials that change with time, sometimes based on 3D printing materials [1]. Therefore, conventional 3D printed materials that can change shape on-demand with time have been termed 4D printed materials, where the fourth dimension is time. In other words, 4D printed materials are commonly known as materials that are the product of a combined strategy blending 3D printed materials (or materials fabricated in other ways) with structural and morphological changes (i.e., shape, physicochemical properties, or functionality) over time [1,2].

The main objective of this study is to develop a facile, adaptable, replicable, biologically functional, and affordable 4D printed material that can be employed for various biomedical applications where a shape-changing property plays a vital role. For instance, these 4D printed materials would find wide use across all of medicine including improved straightening of the curved spine in scoliosis patients where current technologies involve surgery after surgery to insert new spinal rods every time the spine straightens to a certain degree [3]. Further, as people age, various sphincters in the body lose mechanical integrity reducing functionality. For example, the sphincter that separates the stomach from the esophagus mechanically weakens with aging causing acid reflux [4]. Thus, materials which can close and open on-demand can help limit acid reflux. As just another example in which shape change materials can aid medicine, consider that orthopedic tissue (such as the intervertebral disk) grows under increased pressure [5]. Thus, materials which can increase in size on-demand can increase pressure in the disk space to increase tissue growth if sufficient new tissue growth is not occurring. There are countless additional examples where medicine can benefit from 4D printed materials which can change shape on demand.

One additional strong application where 4D printed materials can improve medicine include stem cell delivery. While stem cells were once considered the panacea for all of medicine, stem cell therapies have not met such high expectations due to faulty delivery methods [6]. As just one of many examples, stem cells were once thought to revolutionize Parkinson's disease treatment. Over 1.2 million people in the U.S. are projected to suffer from Parkinson's Disease by 2030 and current therapies on the market offering pharmaceuticals and surgically placed medical devices, such as deep brain stimulators, only address symptoms of Parkinson's disease [7-10]. To replace the lost dopaminergic neurons of the substantia nigra pars compacta in patients with Parkinson's Disease, a tissue transplantation approach has been frequently employed since the 1980s [7-10]; however, there are many problems with such treatments resulting from inadequate stem cell (specifically, autologous induced pluripotent stem cell (iPSC)) isolation, culture, and insertion procedures [7-10]. In particular, for the injection of stem cells into patients, stem cells often die at amounts up to 70% after delivery [7-10]. This is because such cells grown in culture for expansion before injection are typically removed from cell culture plates using harsh enzymes (like trypsin) which disrupt cell to cell contacts, reverse intended differentiation, and overall can lead to cell death. Further, stem cells have been shown to migrate away from the areas of the brain in which they were implanted, migrating away from the damaged tissue in which they are needed the most for repair [7-10]. Thus, inserting stem cells with 4D printed materials can ensure proper stem cell placement in the body.

These 4D printed materials have several advantages over the traditional materials used for stem cell delivery or injection to the site of injury. For instance, regarding cell delivery, 4D printed materials

can be used as the same material to culture cells as that to implant cells avoiding the need for harsh cell lifting chemicals and can also be used to anchor such cells at the diseased site [11]. However, for this to occur, materials are needed which can on-demand change shape from flat for cell culture to tubular for injection then flat again after insertion into tissue for cell delivery. To accomplish these goals, this study developed 4D printed materials which can be fabricated for one of many promising medical applications: improved cell delivery for treating neurological diseases, such as Parkinson's disease. Herein, the protocol for fabricating such 4D printed materials involves straightforward and easy-to-follow steps that firstly require fabrication of a suitable material in which cells are seeded. Then, the 3D (bio)printed material should be heated up to 37 °C for a shape change from flat to tubular for easier loading to a syringe and subsequent injection. This 4D printed material will provide a replicable and low-cost method that allows researchers in most academic research laboratories or industrial settings to form such materials for specific biomedical applications.

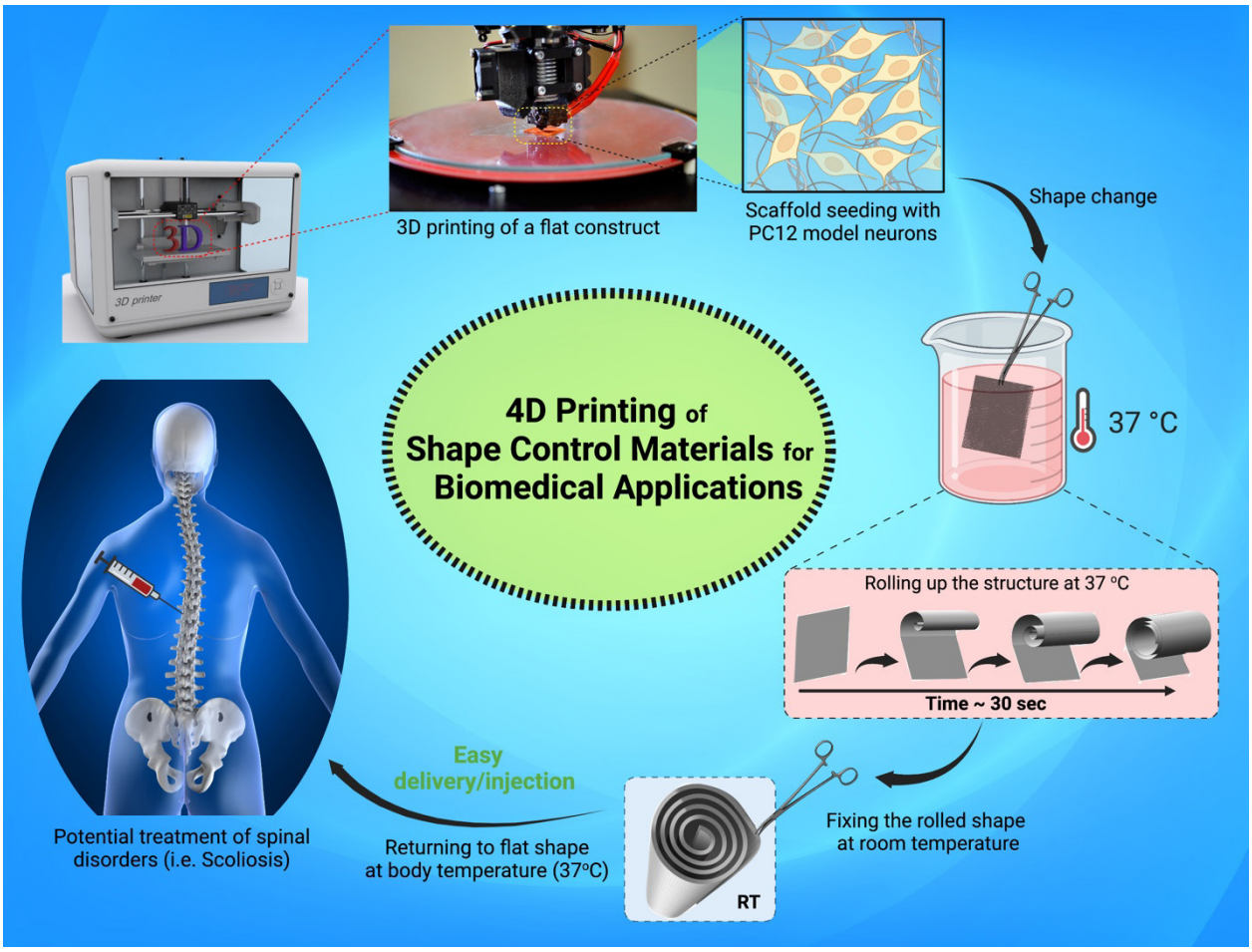
Materials and Methods

All chemicals were obtained from Sigma unless other indicated. 1 ml of a 1.7 g dissolved bisphenol solution was mixed into 122 mL of a 0.17 g dissolved poly (propylene glycol) solution. 500 mL of a 0.58 g dissolved decylamine solution was added to the above solution at 50 °C for 5 min. The mixture was then poured into a glass mold representing the desired shape (1 by 1 by 0.1 cm^3) and allow to cure (solidify) for 48 hours at room temperature. The construct was then detached from the glass mold and transferred for in vitro use under a biosafety culture hood. The construct was sterilized by exposing it for 30 min to UV-radiation under a cell culture laminar flow hood.

Such materials can be used for numerous medical applications, most prominently for delivering cells from *in vitro* culture to *in vivo* studies. For instance, to demonstrate this for neurological applications, this study placed the printed structure in one of the wells of a 6-well plate and seeded 2,000 cells/cm^2 PC-12 neuron-like cells (ATCC) onto a flat version of the 4D printed material (with a 1 by 1 cm^2 surface area) in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The substrate was incubated for 40-45 minutes in a standard cell culture incubator at 37 °C and 5%CO_2 to allow for the attachment of the cells. The construct was removed out of the incubator and under a biosafety cabinet, the old media and unattached cells were removed and 3 ml of culture medium (Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) was added followed by incubation for 1 day under standard incubator conditions (specifically, a humidified, 37 °C, and 95%/5% air/CO_2 environment).

To demonstrate the shape change of the material, when the cell-laden 4D printed flat construct is transferred to 37 °C, it can be molded (while keeping it at 37 °C) into the shape that is desired, such as a tube to serve as a cell delivery device. Specifically, as a proof of concept, the cell-laden construct formed above can be held with tweezers and placed into a water bath heated to emulate body temperature (37 °C). While in water bath, it can be molded it into the shape that is desired (in this study the flat structure was molded into a tubular shape as shown in **Figure 1**), such as a tube to serve as a cell delivery device. For this, all materials should be sterile.

Figure 1. Schematic illustration of 4D Printed Shape Control Material with a Potential Application for the Treatment of Spinal Disorders (i.e., Scoliosis).



The samples were removed from the incubator after 24 h and one can watch it maintain its shape at room temperature in order to, for example, transfer from an in vitro cell culture facility to the clinic to be implanted. The material will be stable at the preformed shape indefinitely at room temperature (for further clarification see schematic **Figure 1**).

In order to show that the material supports cell functions and the 4D shape change does not alter cell functions, after a 24 h incubation, cell viability of the PC-12 cells was examined using MTT assays to determine cell number and the number of neurites per cell [12-15]. Assays were repeated in triplicate and at least three times each.

Then, when ready for implantation, rather than using harmful chemicals, like trypsin, to remove the cells from the bottom of a typical tissue culture polystyrene plate for injection which can result in the death of up to 30-70% of cells[7-10], the shape of the 4D printed material with the cells cultured on its surface could be directly altered into an easy to implant tubular shape by rolling the material at 37 °C.

Upon implantation, the construct will be exposed to body temperature (37 °C) and return its shape to an unfold state which is appropriate for stem cell delivery to the area of need. To confirm this happens after implantation, a simple in vitro experiment can be performed, where the folded cell-laden 4D printed material can be placed back into a heated water bath to emulate body temperature (37 °C), and then watch the folded construct immediately unfold to the original flat shape. For this, all materials should be sterile.

Results

As a proof of concept, this study demonstrated that PC-12 cells (model neurons) can be cultured on a 4D printed cell culture substrate, then the shape of that cell culture substrate can be changed from flat to tubular with the PC-12 cells still on such substrates for their theoretically improved delivery into the spine of patients suffering from scoliosis (or a curved spine) or for any other disease. Clinically, using 4D printed materials in this way maintains cell viability and functions much better than using trypsin to lift cells off of static cell culture plates (which will reduce cell viability and alter cell function[7-10]), placing the cells into a syringe, and injecting them into the defect site. Although requiring more studies, it is anticipated that in an in vivo investigation, the PC-12 cells delivered via the 4D printed would improve spinal tissue regeneration to enable straightening of the spine in a natural manner without repeated surgeries to implant metallic spinal rods as the spine straightens.

Supplementary Video 1 (Video S1) shows the ability to control the shape of the 4D printed material suitable for the cell delivery described above as well as other medical applications. The video can be accessed throught this link: https://www.youtube.com/watch?v=50xT01gkS5U As demonstrated in this video, the 4D construct returns to a flat shape at 37°C in less than 30 s (from 01:02 to 1:22 timeframe in **Video S1**).

Figure 2: The same number of PC-12 cells before and after shape change. Data = mean +/- SEM; N = 3; values are statistically the same.

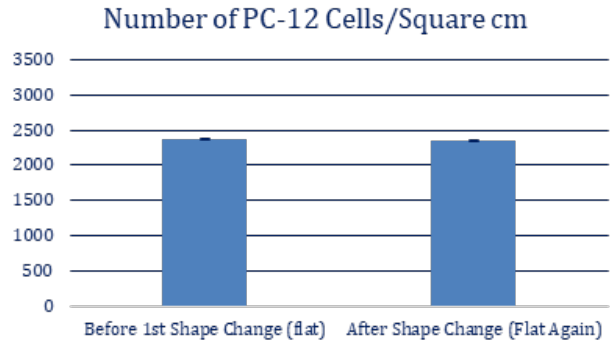


Figure 3: The same number of neurites per PC-12 cells before and after shape change. Data = mean +/- SEM; N = 3; values are statistically the same.

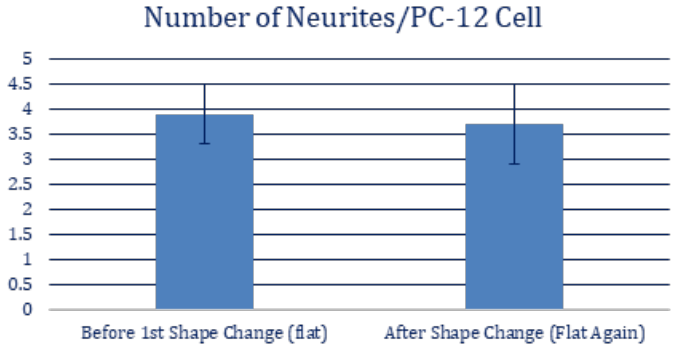


Figure 2 shows that the number of PC-12 cells before and after the 4D material shape change was not altered. **Figure 3** shows that the number of neurites per PC-12 cell before and after 4D material shape change was not altered. **Figures 2 and 3** provide significant promising evidence that cell function is not altered when cultured on the proposed 4D printed materials undergoing a shape change. This is

in contrast to typical cell delivery methods in which up to 70% of cells typically die from enzymatically lifting from tissue culture polystyrene (using chemicals such as trypsin) to loading into a syringe for injection into tissue.

Discussion

This study provides a simple easy-to-follow procedure to formulate a material which can change shape on-demand when exposed to body temperatures: a so-called 4D printed material. As described, three common materials are added to formulate a large or small material which can be molded into any desirable shape for any medical application. Further, this material can be 3D printed or formulated in a mold into a described shape. Results clearly showed the fast ability of this material to change shape when heated, maintain that shape at room temperature, and change back when heated again to body temperature. The 4D printed constructs could remarkably maintain both flat and tubular shapes, and both conformations were structurally stable without any external forces to maintain its structure, so called materials with zero degree-of-freedom (DOF). Further, this study demonstrated that a model cell line (PC-12) attached and grew on the material and the number of PC-12 cells and neurite extensions per neurite did not change when using the material or undergoing a shape change. This highlights the material's cytocompatibility and feasibility of using such a shape change material as an alternative to harsh cell lifting agents (like trypsin) for various cell delivery strategies.

Importantly, there are several critical steps to this procedure. The first is the selection of the 4D printed materials. Not all materials will support PC-12 function, so the proper material must be chosen. The material described within supports PC-12 function. Second, the final dimensions of the construct must match clinical needs. Specifically, if one plans to use a syringe to inert the 4D printed tubular construct, the tubular construct must obviously fit into the syringe and be of an appropriate size for the intended tissue site. The dimensions used here match a clinical application for inserting PC-12 stem cells into a spinal defect. Thus, if using the above material, dimensions, cells, and clinical use, there is no troubleshooting or modifications needed for this protocol.

While the present material is promising, further improvements in the protocol could be made. For example, more complicated self-assembled chemistries exist which have been 3D printed into various shapes and sizes and after incorporating graphene nanoparticles (which heat up when stimulated by near infrared; NIR) can change shape controllably and on-demand [17-23]. Such 4D printed self-assembled materials were not highlighted here due to their significantly more complex synthesis method, however, such NIR control shape change materials provide for greater on-demand shape change than using temperature alone as this study did. Prior work has demonstrated that such self-assembled materials can control drug release over months and can regenerate bone, cartilage, cardiovascular, and other tissues [17-23]. Similar to the present study, prior studies have further highlighted healthy stem cell functions on such self-assembled 4D printed chemistries embedded with graphene nanoparticles under NIR shape control [17].

None-the-less, whether the shape change is stimulated by temperature or NIR, there are numerous future medical applications for these dynamic materials, representing a significant change from today's static medical devices. From treating scoliosis where new spinal rods have to be inserted upon every small straightening of the spine to on-demand increase in pressure to promote bone growth, 4D printed materials should be the cornerstone of all future medicine. Having protocols like those presented here that are easy-to-follow, inexpensive, and simple will help to emphasize the importance of 4D printed materials in improved medicine. This technology is a significant advancement over 3D printing materials which do not change shape.

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Disclosure of Interests

A patent from one of the authors (T.J.W.) outlining this technology has been licensed to form the start-up company Interstellar Therapeutics. Thomas J. Webster is also a member of the editorial board.

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