Biomaterials 140 (2017) 58–68

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

3D printed lattices as an activation and expansion platform for T cell therapy

Bahman Delalat [a, d, 1], Frances Harding [a, d, 1], Batjargal Gundsambuu [b, d], Elena M. De-Juan-Pardo [c, d], Felix M. Wunner [c, d], Marie-Luise Wille [c], Marek Jasieniak [d, e], Kristen A.L. Malatesta [b, d], Hans J. Griesser [d, e], Antonio Simula [d], Dietmar W. Hutmacher [c, d, f, 2], Nicolas H. Voelcker [a, d, 2], Simon C. Barry [b, d, g, *, 2]

[a] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Future Industries Institute, University of South Australia, Adelaide, Australia
[b] Molecular Immunology, Dept. of Gastroenterology, Women's and Children's Hospital, Adelaide, Australia
[c] Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
[d] Cooperative Research Centre for Cell Therapy Manufacturing, Adelaide, Australia
[e] Future Industries Institute, University of South Australia, Adelaide, Australia
[f] ARC Centre in Additive Bio-manufacturing, Queensland University of Technology, Brisbane, Australia
[g] Robinson Research Institute, University of Adelaide, Adelaide, Australia

ARTICLE INFO

Article history:
Received 8 March 2017
Received in revised form
26 April 2017
Accepted 7 May 2017
Available online 7 June 2017

Keywords:
Melt electrospin writing
3D lattice
Plasma polymer functionalization
Immunotherapy
Cell therapy manufacturing

ABSTRACT

One of the most significant hurdles to the affordable, accessible delivery of cell therapy is the cost and difficulty of expanding cells to clinically relevant numbers. Immunotherapy to prevent autoimmune disease, tolerate organ transplants or target cancer critically relies on the expansion of specialized T cell populations. We have designed 3D-printed cell culture lattices with highly organized micron-scale architectures, functionalized via plasma polymerization to bind monoclonal antibodies that trigger cell proliferation. This 3D technology platform facilitate the expansion of therapeutic human T cell subsets, including regulatory, effector, and cytotoxic T cells while maintaining the correct phenotype. Lentiviral gene delivery to T cells is enhanced in the presence of the lattices. Incorporation of the lattice format into existing cell culture vessels such as the G-Rex system is feasible. This cell expansion platform is user-friendly and expedites cell recovery and scale-up, making it ideal for translating T cell therapies from bench to bedside.

Cell therapy is recognized as the next pillar of regenerative medicine, and its clinical impact has already been demonstrated by bone marrow transplants and blood transfusion, procedures routinely performed for many decades [1–3]. A number of recently emerging immunotherapies have broadened the clinical indications for cell therapy. These therapies all rely on *ex vivo* expansion in order to achieve therapeutic doses, so simple, affordable and scalable expansion technologies can be applied to each of these therapies.

One of these recent advances has been the induction of tolerance to organ and cell transplants, including bone marrow, solid organs and islet cells, using regulatory T (Treg) cells, which are identifiable by their expression of CD25 and FOXP3, and absence of CD127 expression [4,5]. Tregs are now well accepted as one of the key immune cells for establishing and maintaining immune tolerance [6,7] and loss of function or reduced numbers of Treg are implicated in a number of autoimmune diseases [8,9]. In addition to applications to treat or reverse autoimmunity [10–12], Treg cells have the potential to achieve transplant tolerance without lifelong immunosuppression—the major cause of mortality and morbidity in transplant patients [13]. However, because activation transiently induces both CD25 and FOXP3 expression in $CD25^{-}$ effector cells, specific controls must be put in place to ensure that the post-expansion product comprises stable Treg cells rather than contaminating activated effector cells [14].

* Corresponding author. Molecular Immunology, Robinson Research Institute, University of Adelaide, and Dept. of Gastroenterology, Women's and Children's Hospital, Adelaide, Australia.
E-mail address: simon.barry@adelaide.edu.au (S.C. Barry).
[1] These authors contributed equally.
[2] These senior authors contributed equally.

http://dx.doi.org/10.1016/j.biomaterials.2017.05.009
0142-9612/

The rapidly emerging cancer immunotherapy field has been enabled by the development of several T cell based cell therapies; Tumor infiltrating lymphocytes (TILs) are isolated from within a solid tumor [15], and are specific to each patient's tumor (reviewed in Ref. [16].) Defined antigen specificity can be induced though expression of clonal T cell receptor genes (TCR-T) or chimeric antigen receptors (CAR-T) [17,18]. CAR-T cells, in particular, are a rapidly emerging technology for cancer immunotherapy (reviewed in Ref. [19]). CAR-T therapy has proved to be extremely effective in phase 1/2 trials, and due to its *in vivo* potency [19–26], but it also requires fewer cells to be delivered than, for example, Treg cell therapy products. Both therapies have the highly desirable attributes of low side-effects and reduced need for adjunct drug therapy, and both can be personalized. The next phase of clinical application will require the manufacture of cells for thousands of recipients to demonstrate safe and effective dosing. The market for these T cell products is predicted to reach US$10 billion by 2020 [27], enabled by implementing technologies that will make these therapies affordable and accessible.

In contrast to other established modalities of cell therapy, these new immunotherapies require extensive cell expansion *ex vivo* to manufacture a clinical product [18]. Arguably one of the biggest hurdles to the widespread delivery of cell-based immunotherapies, such as Treg cell therapy, is the cost of goods (COG) [10–12]. Reducing COG requires simple, affordable and scalable manufacturing processes. To generate the numbers of T cells required for large-scale therapy T cells first need to be isolated to high purity and then expanded *in vitro* under conditions that preserve their phenotype. Expansion protocols exploit the capacity for T cells to proliferate in response to a pathogen challenge as part of a normal immune response. *Ex vivo*, this can be mimicked in a cell- and antigen-independent manner using monoclonal antibodies to CD3 and CD28, presented in combination with appropriate T cell growth factors, such as IL2, IL7 or IL15.

Current practice for clinical scale-up includes the use of antibody-coated cell culture plates or culture in the presence of costly antibody-coated magnetic beads (e.g. Dynal expander beads), the current gold standard of the research community and cell therapy industry [28]. We present an alternative; a modular, scalable, multilayered lattice with highly organized architectures that can simultaneously present the co-stimulatory signals to a large number of cells. Our 3D lattice is manufactured by melt electrospinning writing (MEW), a novel 3D-printing technology that allows the fabrication of highly organized fibrous constructs from micro- to nano-scales under the influence of an applied electrical field [29–31]. MEW structures provide significantly enhanced surface area and structural properties compared with other melt-based 3D-printing technologies, due to smaller fiber diameters and highly ordered architectures. Scaffolds created using MEW have been used in regenerative medicine in which the scaffold and cells together generate the replacement tissue [32–35]. Here, MEW structures are converted to a lattice cell-culture platform that sustains the growth of T cells, with limited cell adhesion. The lattices were coated via plasma polymerization to introduce reactive functional groups for covalent anti-CD3 and anti-CD28 antibody attachment in a conformal coating around the fibers of the 3D lattice [36–41]. The highly organized 3D lattice allows sufficient space for T cell blasts to grow, facilitates nutrient exchange, and enables repeat interactions to further enhance stimulation and expansion of the T cells. We demonstrate the use of this antibody-presenting 3D lattice to expand $CD4^+$ $CD25^+$ Treg cells, $CD4^+$ effector cells and $CD8^+$ T cells, demonstrating robust *in vitro* expansion of highly functionally specific immune cells for clinical immunotherapy. A key feature of the platform, unlike bead based systems, is the ability to recover cells by simply harvesting them from the expansion platform, rather than a separate magnetic based bead removal step. Additionally, functionalized lattices can efficiently facilitate stable lentiviral gene delivery as well as enabling T cell expansion, which is of particular relevance for the clinical manufacture of CAR-T cells. The potential for scalable cell expansion in cGMP conditions suggests that this technology will significantly reduce COG for cell therapy manufacturing, yet maintain and/or improve the functionality of the expanded cells.

1. Methods

1.1. Design and 3D printing of lattices for T cell expansion

1.1.1. Melt electrospinning writing

Highly ordered cell culture lattices were 3D-printed using a customized melt electrospinning writing (MEW) device. We previously described the spinning process and printer [42], but made modifications for this study, as described below. A 5 mL glass syringe (Gastight® Cat. No. 1005, Hamilton Co., USA) was loaded with medical-grade PCL pellets (Purasorb® PC12, Purac Biomaterials, Netherlands). The polymer was heated to 90–100 °C. The syringe was connected to a programmable pump (AL-1000, World Precision Instruments Inc., USA) via a physical adapter to extrude the molten polymer through a 23G Luer lock metal needle at a constant flow rate of 10 μm/h. A high voltage, between 10.5 and 12.5 kV, was applied to the needle (DX250R, EMCO High Voltage Co., US) and a grounded stainless steel collector was positioned at a distance of 20 mm to the needle. When the extruded melted polymer flew through the spinneret, an electrostatically drawn jet was accelerated toward the grounded collector. A motorized XY positioning slide (Velmex Inc., USA) driven by a CNC controller software (Mach3, Newfangled Solutions, USA) was used to displace the collector with programmable patterns to collect lattices of defined fiber spacing and orientation: 200, 500 and 1000 μm fiber spacing with a laydown pattern of 0°/90°. Large lattices in sheet format were produced (120 mm × 120 mm and 150 mm × 150 mm), then cut to size using a laser-cutting machine (ILS12.75, Universal Laser Systems, Inc. USA).

For randomized lattices, the fibres were deposited as described above on the grounded stainless steel collector, but placed in a random manner controlled with the CNC Software. The polymer was molten at 83 °C, the air-pressure was kept at 1.8 bar, and the applied voltage set to 10.5 kV, with a working distance of 17 mm from collector surface to the tip of the needle. The stage was programmed to move in XY in repetitive circles with a radius of 4 mm.

1.1.2. Surface functionalization

Lattices were coated with a plasma polymer to produce an epoxy-functionalized surface. We have previously described the customized plasma rig and its operation[19]. Allyl glycidyl ether (>99%) was obtained from Sigma-Aldrich (USA). The plasma deposition protocol used two steps of plasma polymerization: constant wave (cw) plasma polymerization for 1 min; and pulsed plasma polymerization (DC, i.e. duty cycle 1 ms/20 ms) for 2 min. The pressure of the allyl glycidyl ether monomer during the treatment was at 0.2 Torr; plasma power was 25 W.

After plasma polymerization, lattices were transferred to 48-well tissue culture-treated plates for antibody conjugation. Lattices were incubated in a solution containing anti-human CD3 functional-grade purified antibody (Affymetrix eBioscience, USA) and anti-human CD28 functional grade purified antibody (Becton Dickinson, USA) in Dulbecco's phosphate buffered saline (PBS, Sigma-Aldrich) at 4 °C overnight. The solution used for antibody coating contained 40 μg/mL of each antibody, unless otherwise stated. Lattices were produced using solutions containing 10, 20, 40