Bags versus flasks: a comparison of cell culture systems for the production of dendritic cell–based immunotherapies

Natalie Fekete,1,2 Ariane V. Béland,1 Katie Campbell,2 Sarah L. Clark,2 and Corinne A. Hoesli1

Cancer represents a significant socioeconomic burden, causing 8.2 million deaths worldwide in 2012.1-4 By 2030, this burden is expected to nearly double, growing to 21.4 million cases and 13.2 million deaths.2 In recent years, cell therapy has emerged as a novel, complex, and very promising therapeutic strategy for the treatment of diseases that do not respond to classical pharmaceutical or biopharmaceutical product-based treatments.5,6 Targeting the immune system—and not the cancer itself—represents a paradigm shift in oncology and vaccinology. Similarly, chimeric antigen receptor (CAR)- and T-cell receptor–engineered T cells have resulted in a landslide transformation in immunology and adoptive cell transfer.7 These sophisticated cell

ABBREVIATIONS: APC = antigen-presenting cell; CAR = chimeric antigen receptor; DC(s) = dendritic cell(s); FEP = fluorinated ethylene propylene; Mo-DC(s) = monocyte-derived DC(s); PFA = perfluoralkoxy copolymers; PTFE = polytetrafluoroethylene.

From the 1Department of Chemical Engineering, McGill University, Montreal, Canada; and 2Saint-Gobain Ceramics & Plastics, Inc., Northboro R&D Center, Northborough, Massachusetts.

Address reprint requests to: Corinne A. Hoesli, Department of Chemical Engineering, McGill University, 3610 University Street, Montreal, QC, Canada, H3A 0C5; e-mail: corinne.hoesli@mcgill.ca.

This work was funded by Saint-Gobain Ceramics & Plastics, Inc.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Received for publication August 23, 2017; revision received February 17, 2018; and accepted February 18, 2018. doi:10.1111/trf.14621

© 2018 The Authors Transfusion published by Wiley Periodicals, Inc. on behalf of AABB

TRANSFUSION 2018;58;1800–1813
products are able to reengage the immune system’s anti-
cancer responses, replace damaged tissues, and heal
chronic wounds.8,9 Considering the magnitude of poten-
tial impact, the field of “cancer immunotherapy” was cho-

tered as the “Breakthrough of the Year” by Science
magazine
in 2013.10-12 The global market for cell therapy was valued
at approximately US$2.5 billion in 2012 and is expected to
reach US$8 billion by 2018.5 The advance of improved
technologies in coming years should further reduce pro-
duction costs and enhance clinical efficacy.

Currently available cancer vaccines include cell-
based, protein-based, recombinant live vector (viral or
bacterial)—based and nucleic acid—based vaccines.6,13
Detailed reports summarizing the state of the art and
proposed mechanisms of action of both virus-based and
DNA-based cancer immunotherapy products have been
published recently elsewhere.13-16 Currently, there are
more than 1900 studies investigating the clinical efficacy
of “cancer vaccines” registered with the US National
Institutes of Health website (www.clinicaltrials.gov).
Most of these trials are in the phase I/II testing safety/efficacy stage, with only a small number of trials in phase
III (Table 1). Antitumor vaccination with viable, complex
active ingredients such as cells is a complicated, multi-
step task.17,18 The optimal clinical-scale production plat-
form and culture modalities for guaranteeing an effective
cell product have yet to be established.

Manufacturing and distribution challenges can signifi-
cantly impact the commercial and clinical viability of per-
sonalized medicines such as cell-based immunotherapy
products. Many challenges with cell culture vessels have
recently been described:19-21 In addition, many patients
prefer to be treated without multiple blood draws and
exposure to pathogens.22-24 Table 1 lists ongoing trials in
which DCs are used for cancer immunotherapy.

APC-BASED CANCER VACCINES

The development of cell-based cancer vaccines originated
with the concept of exploiting the potential of APCs to
trigger the immune system. APCs are uniquely qualified
to initiate a specific and targeted immune reaction against
the presented tumor-associated antigens.17-19 In vivo, DCs
act as the “professional” and most potent APCs of the
immune system.20 The antigens used in cancer vaccines
are typically derived either from the patient’s own tumor
or are presented to the cells as a recombinant tumor-
associated antigen, which may be fused to an adjuvant
protein for co-delivery.16

Generating tumor-specific APCs in vitro

In vitro, several cell types can be used as progenitor cells
to obtain APCs that are phenotypically similar to DCs.21-25
Peripheral blood mononuclear cells (PBMCs) and CD34+
 hematopoietic progenitor cells have been used as sources
for DC generation, but monocytes remain the most com-
monly used progenitor cell type.26-30 Monocytes can be
induced to generate DCs via addition of differentiation-
inducing cytokines, typically interleukin (IL)-4 and
granulocyte-macrophage colony-stimulating factor,31,32
during 3 to 7 days in culture.33

Cell maturation can be achieved via the addition of a
cocktail of inflammatory cytokines and/or toll-like receptor
agonists, such as tumor necrosis factor-α, prosta-
glandin E2, IL-6, lipopolysaccharide and polyinosinic:poly-
cytidylic acid in the final 2 days of culture.34,35 During this time period,
patient-specific tumor-associated antigens may be added to

| Study start date | Sponsor | Target indication | Product ID |
|-----------------|---------|------------------|------------|
| 2016            | Radboud University | Melanoma | nDC: natural DCs | NCT02993315 |
| 2014            | Sotio a.s. | Metastatic castrate-resistant prostate cancer | DCVAC/PCa: autologous DCs | NCT02111577 |
| 2014            | University Hospital, Erlangen, Germany | Uveal melanoma | Autologous DCs loaded with autologous tumor RNA | NCT01983748 |
| 2012            | Argos Therapeutics, Inc. | Renal cell carcinoma | AGS-003: autologous DC immunotherapy | NCT01582672 |
| 2006            | Northwest Biotherapeutics, Inc. | Glioblastoma multiforme | DCVax-L: autologous DCs pulsed with tumor lysate antigen | NCT00045968 |

* DC immunotherapy studies listed in the clinicaltrials.gov registry as Phase 3 “Recruiting” or “Active, not recruiting” with the search term “dendritic cell” accessed on December 28, 2017.
the cultures for uptake by the DCs. Following maturation, DCs are then administered to the patient, usually in a series of repeated injections. The maturation phase of the DCs remains less standardized, providing an opportunity for optimization and patient tailoring of the manufacturing system. To this end, both biomedical (generation of DCs and their appropriate stimulation) and engineering (selection of culture vessels and microenvironment) considerations should be taken into account.

**Sipuleucel-T**

Sipuleucel-T was the first and so far only Food and Drug Administration–approved cell-based cancer vaccine. This product was developed by Dendreon Corp. Sipuleucel-T is intended as a first-line treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer in patients that no longer respond to conventional hormone therapy. Sipuleucel-T is generated from the patient’s own peripheral blood mononuclear cells, which are subsequently enriched for APCs and exposed to a fusion protein comprised of prosthetic acid phosphatase and granulocyte macrophage colony-stimulating factor. The cells are reinfused into the patients within 3 days of the initial leukapheresis. The efficacy of this cell-based immunotherapy was first reported in the landmark phase III IMPACT trial. There, patients received a total of three infusions of the APCs generated in vitro using a fully closed fluoropolymer cell culture bag system. Patients treated with sipuleucel-T had a 4-month overall survival benefit compared to patients infused with the placebo. Despite this and other recent achievements, the precise immunologic mechanisms of action underlying this therapeutic effect are challenging to assess and therefore still unclear.

**DC cultures: Suspension versus anchorage-dependent systems**

Many traditional monocyte-derived DC (Mo-DC) culture protocols rely on the adherence of both monocytes and DCs to their culture substrates. Several protocols use the plastic adherence of monocytes in an initial enrichment step before removing all nonadherent cells and proceeding to culture only the adherent cells as APCs. This implied anchorage dependency of Mo-DCs in vitro contrasts with more recent protocols eschewing adherent cells for DCs cultured in suspension. Rouard and colleagues cultured monocytes in hydrophobic bags before transferring them to polystyrene cultures for their maturation as adherent cells. Coulon and colleagues, on the other hand, first cultured monocytes as adherent cell cultures on polystyrene surfaces before transferring them into suspension cultures in fluorinated ethylene propylene (FEP) bags at Day 5 for DC culture. An interesting approach proposing Mo-DC production in a novel closed-cell culture system utilizing a combination of hydrophobic cell culture bags and styrene copolymer microcarrier beads was proposed by Maffei and colleagues.

**Closing the manufacturing process: Switching Mo-DC culture from polystyrene to fluoropolymer surfaces**

Polystyrene-based flask or multiwell cultures

Polystyrene has been traditionally used for cell culture since the 1960s. To support the culture of anchorage-dependent cells, the hydrophobic polystyrene must usually be surface treated to render it more hydrophilic and thereby facilitate cell adhesion and spreading in culture conditions. This treatment is proprietary to each commercial manufacturer, but reportedly typically consists of a plasma treatment, which produces additional hydroxyl, carboxyl, and aldehyde groups on the culture surface. Battiston and colleagues compared tissue culture polystyrene surfaces from three different companies (Sarstedt, Wisent Corp., and Becton Dickinson) and found marked differences in protein adsorption, surface wettability, and monocyte retention following 7 days of culture. This study and others underline the frequently underreported...
### TABLE 2. Studies comparing flask to bag culture systems for DC immunotherapy

| Reference | Bag system vs. Flask, multiwell system | Culture medium; supplements | Maturation factors | Potency assays | Outcome |
|-----------|----------------------------------------|-----------------------------|--------------------|----------------|---------|
| Macke, 2010<sup>66</sup> | Polyolefin bags vs. Polystyrol-based cell culture flasks | CellGro (CellGenix); GM-CSF and IL-4 | IL-1b, IL-6, TNF-α and PGE2 (48 h) | FC, SEM, MLR, DNA Microarray | No objective difference in the DCs generated in both systems |
| Tan, 2008<sup>69</sup> | Polyolefin bags vs. Surface-treated polystyrene flasks | CellGro; GM-CSF, IL-4, TNF-α and 10% autologous plasma | N/A | FC, allogeneic MLR | Reduced fraction of DCs in bags (4.7%) compared to flasks (40%) based on flow cytometry |
| Kurlander, 2006<sup>70</sup> | FEP bags vs. Polystyrene flasks | RPMI 1640 with autologous plasma or human AB serum; GM-CSF and IL-4 | CD40L and IFN-γ or poly(I:C) and IFN-γ (24 h) | FC, ELISA, DC migration assay, IFN-γ production of autologous T cells | FEP- and PS-cultured DCs are similar in phenotype and in some functional measures, but FEP markedly reduces DC production of IL-12 and IL-10 |
| Elias, 2005<sup>60</sup> | Polyolefin bags vs. Polystyrene multiwell plates | CellGro DC for bags, X-VIVO + autologous serum for plates; GM-CSF and IL-4 | LPS or TNF-α, IL-1b, IL-6 and PGE2 (48 h) | FC, MLR, autologous antigen presentation | No difference in viability; comparable phenotype with exception of CD1a |
| Wong, 2002<sup>67</sup> | FEP bags vs. Polystyrene flasks | X-VIVO 15; GM-CSF and IL-4 | None | FC, MLR, autologous recall responses to tetanus toxoid and influenza virus antigen presentation | DCs were equivalent in yield, phenotype, and in vitro function |
| Guyre, 2002<sup>71</sup> | Polyolefin bags vs. Polystyrene flasks | AIM V; GM-CSF and IL-4 | R848 or TNF-α (24-72 h) | FC, MLR, autologous antigen presentation | Bag-cultured DCs were superior to flask-cultured cells in terms of yield, viability, and function |
| Suen, 2001<sup>72</sup> | FEP bags vs. Polystyrene T175 flasks | X-VIVO 15; GM-CSF and IL-4 | None | FC, MLR, dextran-uptake assay | DCs have similar viability, purity, phenotype, yield, and function |

ELISA = enzyme-linked immunosorbent assay; FC = flow cytometry; FEP = fluorinated ethylene propylene; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; MLR = mixed lymphocyte reaction; PGE2 = prostaglandin-E2; R848 = resiquimod; TNF = tumor necrosis factor.
observation that the choice of tissue culture polystyrene from different manufacturers can lead to significant disparities in terms of protein adsorption and cell spreading characteristics.\textsuperscript{79-81} Given the ubiquitous presence of proteins in animal cell culture media, protein adsorption likely plays a key role in determining cell adhesion to surfaces. Cell surface adhesion is in fact thought to be largely mediated by proteins adsorbed to the surfaces, rather than the surfaces themselves.\textsuperscript{74,82}

**Polyolefin- and fluoropolymer-based bag cell culture systems**

In contrast to the more rigid polystyrene-based cell culture plastics such as multiwell plates or T-flasks commonly found in most cell culture laboratories, cell culture bags are made of more flexible polymers, including polyolefins and fluoropolymers. The materials of fabrication of examples of closed systems used to scale up DC production are listed in Table 3. The most commonly known examples of polyolefins include polyethylene and polypropylene. Polyolefin-based cell containers have been tested for both in vitro culture and storage of CD34+ cells, platelets, DCs, and T cells.\textsuperscript{60,66,67,72,84-86} In the case of monocyte-derived APCs, different monocyte isolation methods may alter the composition of “contaminating” cell populations and proteins entrained with the monocytes seeded, which may in turn impact the propensity of monocytes to adhere to surfaces and to differentiate into APCs.

**TABLE 3. Examples of closed culture systems used for DC production**

| Material of fabrication | Format | Approximate standard capacity range (cm\(^2\))* | Examples of commercial systems and manufacturers (currently available or used in published DC culture studies) |
|------------------------|--------|-----------------------------------------------|--------------------------------------------------------------------------------------------------|
| Polystyrene            | Layered cell culture vessels | 600-60,000 | HYPERStack (Corning), Cell Factory (Nunc Thermo Scientific) |
| Silicone membrane bottom | Bottle with membrane bottom | 50-500 | G-Rex |
| Polyolefins            | Bags   | 20-200 | CellGro Cell Expansion Bags (Mediatech Corning), EXP-Pak Bio-Containers (Charter Medical), MACS GMP Cell Expansion Bag (Miltenyi Biotec), LifeCell X-Fold Cell Culture Container, Opticyte, (all Nexell, Baxter Healthcare; discontinued) |
| Fluoropolymers (e.g., FEP) | Bags | 20-5000 | VueLife (Saint Gobain), PermaLife (OriGen Biomedical), SteriCell (DuPont; discontinued) |
| PVC, EVA, and other polymers or copolymers | Bags | 100-1000 | Evolve (OriGen Biomedical) |

* The typical working volume is 0.2-0.5 mL/cm\(^2\) for flasks or layered vessels, 4-11 mL for the membrane-bottom vessel system, or 0.5-1 mL/cm\(^2\) for bags.
† Made from PL732 (polyolefin) coextruded with PL705 (high-impact polystyrene) on the inner surface.\textsuperscript{83}
‡ Formerly manufactured by American Fluoroseal Corporation.
EVA = ethylene-vinyl acetate; FEP = fluorinated ethylene propylene; PVC = polyvinyl chloride.

**Fig. 1. Cellular microenvironment in vitro.** During culture, cells interact with their microenvironment via a plethora of membrane-bound proteins. They are exposed to a multitude of physical, chemical, and biological cues including soluble (signaling or growth) factors in the culture medium, the protein adlayer adsorbed to the culture surface and other cells. [Color figure can be viewed at wileyonlinelibrary.com]
Fluoropolymers are a family of high-performance plastics consisting of fully or partially fluorinated monomers. Many of these polymers are linear and with a backbone of carbon-carbon bonds and pendant carbon-fluorine bonds. The carbon-fluorine bonds of fluoropolymers directly correlate to the unique physical and chemical properties associated with fluoropolymers including low surface free energy and coefficient of friction, chemical inertness, excellent electrical properties, high thermal stability, and maintenance of physical properties even at cryogenic temperatures—a combination of properties that make them interesting candidates for biomedical applications.\(^87\)

Polytetrafluoroethylene (PTFE) was the first fluoropolymer, discovered in 1938 by Dr. Roy Plunkett of DuPont, and was marketed under the brand name Teflon.\(^88\) Commonly used fluoropolymers today include the perfluoropolymers PTFE, FEP, and perfluoroalkoxy copolymers (PFA) as well as partially fluorinated materials such as ethylene tetrafluoroethylene and polyvinylidene fluoride.\(^89,90\) PTFE, FEP, and PFA are all sold under the Teflon trademark from Chemours. PTFE is also sold under the Polyflon trademark and FEP and PFA under the Neoflon trademark from Daikin.

Fluoropolymers have found extensive commercial application in the chemical, electronic, automotive, and construction industries and, with the exception of PTFE, are melt-extrudable thermoplastics that can be processed using traditional polymer processing methods.\(^89,90\)

Similar to polyolefin-based bag systems, fluoropolymer-made bags are transparent, flexible, and permeable to oxygen, nitrogen, and carbon dioxide, allowing gas exchange in cell culture incubators. Fluoropolymers are highly resistant to almost all aggressive chemicals and biologics and remain flexible at temperatures ranging from \(-240^\circ C\) to \(+205^\circ C\). Melt-processable fluoropolymers such as FEP and PFA offer an excellent alternative to polystyrene containers because of their thermal stability across a wide range of temperatures and the ability to be processed using melt extrusion or thermoforming methods.\(^91,92\) Certain fluoropolymer bags are also suitable for cell cryopreservation.

**BIOPROCESSING AND SCALE-UP CONSIDERATIONS IN BAG CULTURE SYSTEMS**

Translating cell cultures to clinical scale

Manufacturing cells for immunotherapy at a clinical scale has traditionally been performed in “open” polystyrene-based vessels both for adherent or suspension cell cultures. Open cell culture systems require the “opening” of flasks or plates for media changes and other cell culture manipulations.\(^93,94\) To decrease the risk of product contamination and by individual operators and to facilitate the scale-up or scale-out and automation of the cell production, “closed” culture systems are thus generally preferred by regulatory authorities to comply with current good manufacturing standards.\(^23,33,42\)

Scaling up flask-based cultures often starts with T-flasks and then progresses to layered polystyrene culture systems, usually using up to eight units in one incubator per patient.\(^95\) Closed or “functionally closed” culture systems that facilitate scale-up have been developed for certain cell types and applications of interest, such as Mo-DC production (Table 3), CAR–modified T cells, or mesenchymal stem/stromal cells for tissue regeneration or graft-versus-host disease therapy.\(^95,96\) The implementation of current good manufacturing practices–compliant closed systems such as bioreactor bags reduces the risks of contamination and improves the overall connectivity between different units of the cultures at an industrial scale. Important considerations when transitioning from open systems...
such as polystyrene flasks to bag-based closed systems are summarized in the following paragraphs and in Fig. 2, including mass transfer, handling, and processing.

Mass transfer considerations

The culture surface area in most commonly used tissue culture flasks ranges from 25 cm² to 225 cm². The recommended fill volume typically corresponds to a liquid height of 2 to 3 mm. This gas/liquid interface is a limiting factor in the scale-up of cell culture systems, as gas exchange almost exclusively occurs via the vented screw cap of the flask. To maintain this level of medium in the presence of evaporation, frequent media changes are necessary, which in turn increases costs of reagents and labor and the probability of contamination. Thus, a variety of gas-permeable bag cell culture containers with low water evaporation rates have been made available to the market sizes up to approximately 2 L fill volume (Table 3). In addition, these devices allow mass transfer through both the upper and the lower surface area of the bags.

The choice of plastic for a closed cell culture system, in which product is not exposed to the room environment, has a significant impact on mass transfer of oxygen, carbon dioxide, and water. As can be seen in Fig. 3, typical plastics used in life science applications have gas permeability and water permeability constants that span three orders of magnitude. Permeability constants are affected by two major materials science parameters: the free volume of a polymer and the chemical compatibility between the polymer and the permeant (e.g., oxygen or water). The free volume of a polymer is the molecular “space” between polymer chains. The effect of the free volume can be directly observed in Fig. 3 by comparing high-density polyethylene (HDPE) and low-density polyethylene. Manufacturers of polyethylene manipulate the free volume of their polyethylene resins by controlling the number and length of branches of the main polyethylene chain during synthesis. Low-density polyethylene has more and longer branches, leading to more free volume and, consequently, higher gas and water permeability than high-density polyethylene. Rubbers are also defined by their higher free volume and tend to have high permeability constants. The impact of chemical compatibility can be observed in Fig. 3 when considering the water permeability constants. Water is a polar solvent and is more chemically compatible with polar plastics such as ethylene-vinyl acetate, leading to higher permeability values. The presence of plasticizers and other fillers can also affect permeability and water vapor transmission through a polymer matrix as shown in Fig. 3 for polyvinyl chloride versus plasticized polyvinyl chloride. In instances where a closed cell culture system requires a plastic material with poor permeability, sterilizing-grade vent filters can be used, as typically employed in polystyrene T-flasks.

Leachables and extractables

In shifting from polystyrene-based flask cultures to bag systems, the profile of leachables and extractables is expected to change considerably due to the different types...
and quantities of plasticizers used in the manufacture of “plastic” disposable systems for cell culture. Leachables and extractables found in polystyrene or ethylene-vinyl acetate polymers can have unknown, and both negative or positive effects on cell processing.107,108 Because extractables’ profiles may vary from lot to lot for some materials, extractables can reduce cell culture process reproducibility. FEP is extruded as a virgin resin and does not contain any additives or plasticizers. As a fully fluorinated polymer, it has very high inherent stability, and there are no modifiers or other content to leach out in water or other solvents. Consequently, extractables are typically at or below detection limits for FEP.

Effect of culture surfaces on cell fate in vitro

The effect of culture surfaces on cell fate during an extended period of time ex vivo is still largely unknown. Several reports document the loss of progenitor cells and cellular properties related to a cell’s “stemness” on planar surfaces displaying nonphysiologic properties.109-111 Interestingly, Yang and colleagues112 showed that stem cells not only respond to mechanical cues presented to them by their microenvironment, but also possess a mechanical memory that plays a role in governing cell fate decisions.113 Polystyrene, the most commonly used material for cell culture vessels, has an elastic modulus of approximately 3 GPa, more than five orders of magnitude stiffer than most cell types.110,114 The FEP used in fluoropolymer bags such as those manufactured by Saint-Gobain has an elastic modulus of approximately 500 MPa. By comparison, the elastic modulus experienced by cells in situ in most tissues is four to six orders of magnitude lower.111,114,115 In Fig. 4, the stiffness of materials commonly used to fabricate cell culture vessels is compared to the stiffness of DCs and other cells or tissues DCs may interact with in vivo.

In addition to mechanical properties, the chemical makeup and wettability of culture surfaces directly impact protein adsorption to surfaces and cell-surface
interactions. In general, in the presence of proteins present in blood and most cell culture media (e.g., insulin, transferrin, albumin), anchorage-dependent cells attach more readily to hydrophilic materials.\textsuperscript{121} The extent of cell adhesion and spreading depends on the type and on the conformation of proteins present on surfaces—a topic that has been extensively reviewed elsewhere.\textsuperscript{122} Cell culture vessel surfaces can be surface treated, typically using plasma treatments, to introduce charged functional groups on surfaces, increase surface hydrophilicity, and promote cell adhesion. For example, polystyrene flasks as well as FEP bags are available in “untreated” (hydrophobic) or “treated” (more hydrophilic) versions. Cell culture vessels can also be preincubated with media containing extracellular matrix proteins that mediate cell adhesion. Extracellular matrix precoating of culture vessels can improve antigen uptake\textsuperscript{123} and/or maturation\textsuperscript{124} of Mo-DCs.

**Studies comparing Mo-DC cultures in bag versus flask systems**

Most studies comparing Mo-DC cultures in polystyrene flasks (adherence) to hydrophobic bags (suspension) report no marked difference between DCs generated in either system (Table 2). Kurlander and colleagues\textsuperscript{70} reported that DCs generated in FEP bags yielded cells with a surface marker expression profile that was comparable to adherence cultures on polystyrene surfaces. However, the DCs generated in the FEP bags produced significantly less IL-10 and IL-12 during their maturation, and these differences persisted upon rechallenge after harvest.\textsuperscript{70} Elias and colleagues reported that after 6 days of culture in polyolefin coextruded with polystyrene cell culture containers (Opticyte, Baxter International, Inc.; see Table 2), DCs no longer expressed CD1a, although they otherwise exhibited a surface phenotype that was comparable to DCs cultured on polystyrene surfaces. These results confirmed a previous observation by Thurner and colleagues.\textsuperscript{53,60} Furthermore, Guyre and colleagues\textsuperscript{71} reported that DC cultured in hydrophobic bags (polyolefin coextruded with polystyrene; see Table 2) were phenotypically different from those cultured in flasks. However, this did not affect their capacity to present antigens, as expression of both major histocompatibility complex class I and class II molecules was consistently high. While the implications of these findings have yet to be determined, these minor differences should be considered when selecting the culture vessel for immunotherapy. Not all cell container systems may be equally suited for generating the optimal cell-based product targeting a specific clinical indication. Stringent potency assays will be necessary to define the essential quality control and release criteria ensuring the generation of efficacious cell therapy products.

In addition, not all cells cultured in hydrophobic bags remain in suspension. For example, Kurlander and colleagues\textsuperscript{70} reported that some Mo-DCs adhered to the surfaces of FEP bags. However, these cells were not used for their analyses, as they represented only a minor percentage of all cultured cells. Guyre and colleagues\textsuperscript{71} performed mixed lymphocyte reaction assays in either polystyrene or polypropylene round-bottom multiwell plates to determine if cell adherence was necessary for DC function, but found only slightly higher proliferative responses in polystyrene wells compared to polypropylene. These observations and the large variety of Mo-DC culture protocols present a yet unsolved conundrum: Is cell adherence during culture beneficial for an efficient differentiation and maturation of Mo-DCs? From a bioprocessing and handling perspective, suspension cultures are preferred when compared to adherence cultures. Moreover, are the adherent cells mature DCs or do they present a different cell type, such as monocyte-derived macrophages? Systematic studies characterizing DCs obtained via adherent and suspension cell cultures using different bag containers will be necessary to investigate the identity and function of the cell products generated within these systems.

**OUTLOOK: HOW TO IMPROVE CLINICAL EFFICACY OF CELL-BASED IMMUNOTHERAPY PRODUCTS?**

The approval of sipuleucel-T by the Food and Drug Administration in 2010 was recognized as an important proof of concept that paved the way for industrial production of cell-based cancer vaccines.\textsuperscript{12} Patients infused with sipuleucel-T demonstrated a significant increase in median survival time by 4 months compared to placebo controls.\textsuperscript{45} However, no differences in the time to disease progression were observed. In addition, no tumor regression or reduction in tumor burden could be measured in treated patients, resulting in novel investigations of the mode and mechanism of action of sipuleucel-T.\textsuperscript{14} Recent findings demonstrated that patients receiving sipuleucel-T showed increased levels of secondary self-antigens, an immunologic response known as antigen spread.\textsuperscript{125} Several approaches are being investigated to achieve the full potential of DC-based vaccines, including increasing the potency of the DCs, targeting the DCs to the tumors, and inhibiting endogenous mechanisms that limit tumor-specific immune responses.\textsuperscript{126} Recent findings also raised questions related to the migratory capacities of the administered APCs to home toward the lymph nodes, a crucial step for the efficient activation of CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes. Reportedly, only 5% of the injected cells reached the lymph node, which may have contributed to the suboptimal success of pioneer immunotherapy products.\textsuperscript{126} The reasons for this impaired homing capacity remain unclear. However, the type, number, and source used for APCs, as well as the site and frequency of injection, may be cornerstones...
The combination of conventional treatments with novel products such as cell-based therapeutics may augment current strategies for patient healthcare. Currently, a wide range of cell types, including hematopoietic stem cells, multipotent progenitors, and fully differentiated effector cells, is manufactured and tested for cell therapy applications. The results of many recent clinical trials, however, remain ambivalent as to the efficacy and potency of these cell products. Additive or synergistic effects between the administered cell products and existing drug-based therapies that lead to desirable clinical outcomes are being explored, but the mechanisms that may lead to these effects remain little understood. Researchers have only recently started to explore the impact of changing cell culture “plastics” for clinical-scale production of cell therapy products. Differences in mass transfer and mechanical and chemical properties can have drastic effects on cell fate. Selecting the appropriate cell cultivation system is thus fundamental in the design and development of cell production strategies. Although this review focused on studies in the immunotherapy field, other therapeutic cell types are being manufactured in the same or similar culture vessels. The handling, processing, and material properties considerations presented in this review will impact the outcome of other therapeutic cell manufacturing processes. Strategically designed cell culture systems will pave the way for the generation of potent cell-based cancer vaccines and other therapeutic cell types.

ACKNOWLEDGMENTS

We thank Dr Jian L. Ding and Mrs Natasha Boghosian at Saint-Gobain for useful discussions and for critically reviewing the manuscript.

REFERENCES

1. Cancer Research UK. Worldwide cancer statistics. 2012. Available from http://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer
2. Cancer.org. Cancer facts & figures 2015. Available from http://www.cancer.org/research/cancerfactsfigures/cancerfactsfigures2015/index
3. Werner M RM, West E. Regenerative medicines: a paradigm shift in healthcare. 2011. Available from http://www.ddw-online.com/personalised-medicine/p142741-regenerative-medicines-a-paradigm-shift-in-healthcare-spring-11.html
4. Torre LA, Siegel RL, Ward EM, et al. Global cancer incidence and mortality rates and trends—an update. Epidemiol Biomarkers Prev 2016;25:16-27.
5. PRWeb. The global cell therapy market to grow at a high rate of 20-22% by 2018, says a new report at ReportsnReports.com. 2014. Available from http://www.virtual-strategy.com/2014/03/06/global-cell-therapy-market-grow-high-rate-0000-0000-0000-says-new-report-reportsnreportscom#P5hw2H8MJ72x5QIE.99
6. Goldman B, DeFrancesco L. The cancer vaccine roller coaster. Nat Biotechnol 2009;27:129-39.
7. Nellan A, Lee DW. Paving the road ahead for CD19 CAR T-cell therapy. Curr Opin Hematol 2015;22:516-20.
8. Sharma P, Wagner K, Wolchok JD, et al. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. Nat Rev Cancer 2011;11:805-12.
9. Ibrahim AM, Wang Y, Lemoine NR. Immune-checkpoint blockade: the springboard for immuno-combination therapy. Gene Ther 2015;22:849-50.
10. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science 2013;342:1432-3.
11. Pardoll D. Immunotherapy: it takes a village. Science 2014;344:149
12. BioMedTracker. Cancer Immunotherapies. 2014. Sagient Research, Inc. May 2014 Report. www.biomedtracker.com.
13. Bolhassani A, Safaiyan S, Rafati S. Improvement of different vaccine delivery systems for cancer therapy. Mol Cancer 2011;10:3
14. Geary SM, Salem AK. Prostate cancer vaccines: update on clinical development. Oncoimmunology 2013;2:e24523
15. Postow M, Callahan MK, Wolchok JD. Beyond cancer vaccines: a reason for future optimism with immunomodulatory therapy. Cancer J 2011;17:372-8.
16. Vergati M, Intrivici C, Huen NY, et al. Strategies for cancer vaccine development. J Biomed Biotechnol 2010;2010.
17. Melero I, Gaudernack G, Gerritsen W, et al. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014;11:509-24.
18. Di Lorenzo G, Buonerba C, Kantoff PW. Immunotherapy for the treatment of prostate cancer. Nat Rev Clin Oncol 2011; 8:551-61.
19. Anassi E, Ndefo UA. Sipuleucel-T (provenge) injection: the first immunotherapy agent (vaccine) for hormone-refractory prostate cancer. PT 2011;36:197-202.
20. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007;449:419-26.
21. Chapuis F, Rösenzwieg M, Yagello M, et al. Differentiation of human dendritic cells from monocytes in vitro. Eur J Immunol 1997;27:431-41.
22. Conti L, Cardone M, Varano B, et al. Role of the cytokine environment and cytokine receptor expression on the generation of functionally distinct dendritic cells from human monocytes. Eur J Immunol 2008;38:750-62.
23. Sorg RV, Ozcan Z, Brefort T, et al. Clinical-scale generation of dendritic cells in a closed system. J Immunother 2003;26:374-83.
24. Lee JJ, Takei M, Hori S, et al. The role of PGE(2) in the differentiation of dendritic cells: how do dendritic cells influence T-cell polarization and chemokine receptor expression? Stem Cells 2002;20:448-59.
25. Dauer M, Obermaier B, Herten J, et al. Mature dendritic cells derived from human monocytes within 48 hours: a novel strategy for dendritic cell differentiation from blood precursors. J Immunol 2003;170:4069-76.
26. Siena S, Di Nicola M, Bregni M, et al. Massive ex vivo generation of functional dendritic cells from mobilized CD34+ blood progenitors for anticancer therapy. Exp Hematol 1995;23:1463-71.
27. Klingemann HG. Immunotherapy with dendritic cells: coming of age? J Hematother Stem Cell Res 2000;9:127-8.
28. Ferlazzo G, Wesa A, Wei WZ, et al. Dendritic cells generated either from CD34+ progenitor cells or from monocytes differ in their ability to activate antigen-specific CD8+ T cells. J Immunol 1999;163:3597-604.
29. Kodama A, Tanaka R, Saito M, et al. A novel and simple method for generation of human dendritic cells from unfractionated peripheral blood mononuclear cells within 2 days: its application for induction of HIV-1-reactive CD4(+) T cells in the hu-PBL SCID mice. Front Microbiol 2013;4:292.
30. Strioga MM, Felzmann T, Powell DJ, Jr, et al. Therapeutic dendritic cell-based cancer vaccines: the state of the art. Crit Rev Immunol 2013;33:489-547.
31. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. Proc Natl Acad Sci U S A 1996;93:2588-92.
32. Mallon DE, Buck A, Reece JC, et al. Monocyte-derived dendritic cells as a model for the study of HIV-1 infection: productive infection and phenotypic changes during culture in human serum. Immunol Cell Biol 1999;77:442-50.
33. van den Bos CKR, Schirmaier C, McCaman M. Pillars of regenerative medicine: therapeutic human cells and their manufacture. Hoboken (NJ): John Wiley & Sons; 2014.
34. Castiello L, Sabatino M, Jin P, et al. Monocyte-derived DC maturation strategies and related pathways: a transcriptional view. Cancer Immunol Immunother 2011;60:457-66.
35. Jamjak-Jankovic S, Hammerstad H, Saeboe-Larssen S, et al. A full scale comparative study of methods for generation of functional dendritic cells for use as cancer vaccines. BMC Cancer 2007;7:119
36. Toungouz M, Quinet C, Thille E, et al. Generation of immature autologous clinical grade dendritic cells for vaccination of cancer patients. Cytotherapy 1999;1:447-53.
37. Tan SM, Kapp M, Flechsig C, et al. Stimulating surface molecules, Th1-polarizing cytokines, proven trafficking—a new protocol for the generation of clinical-grade dendritic cells. Cytotherapy 2013;15:492-506.
38. Lee HJ CN, Yo MC, Hoang MD, Lee YK, et al. Generation of multiple peptide cocktail-pulsed dendritic cells as a cancer vaccine. New York: Humana Press; 2014.
39. Tsujimoto H, Efron PA, Matsumoto T, et al. Maturation of murine bone marrow-derived dendritic cells with poly(I:C) produces altered TLR-9 expression and response to CpG DNA. Immunol Lett 2006;107:155-62.
40. Rouas R, Lewalle P, El OE, et al. Poly(I:C) used for human dendritic cell maturation preserves their ability to secondarily secrete bioactive IL-12. Int Immunol 2004;16:767-73.
41. Dearman RJ, Cumberbatch M, Maxwell G, et al. Toll-like receptor ligand activation of murine bone marrow-derived dendritic cells. Immunology 2009;126:475-84.
42. Van den Bos CKR, Schirmaier C, McCaman M. Therapeutic human cells: manufacture for cell therapy/regenerative medicine. New York: Springer; 2014.
43. Drake CG, Sharma P, Gerritsen W. Metastatic castration-resistant prostate cancer: new therapies, novel combination strategies and implications for immunotherapy. Oncogene 2014;33:5053-64.
44. Dawson NA, Roesch EE. Sipuleucel-T and immunotherapy in the treatment of prostate cancer. Expert Opin Biol Ther 2014;14:709-19.
45. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
46. Di Lorenzo G, Ferro M, Buonera C. Sipuleucel-T (Provenge(R)) for castration-resistant prostate cancer. BJU Int 2012;110:E99-104.
47. Pickl WF, Majdic O, Kohl P, et al. Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. J Immunol 1996;157:3850-9.
48. Romani N, Reider D, Heuer M, et al. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. J Immunol Methods 1996;196:137-51.
49. Tarte K, Fiol G, Rossi JF, et al. Extensive characterization of dendritic cells generated in serum-free conditions: regulation of soluble antigen uptake, apoptotic tumor cell phagocytosis, chemotaxis and T cell activation during maturation in vitro. Leukemia 2000;14:2182-92.
50. Toujas L, Delcros JG, Diez E, et al. Human monocyte-derived macrophages and dendritic cells are comparably
effective in vitro in presenting HLA class I–restricted exogenous peptides. Immunology 1997;91:635-42.
51. Brossart P, Wirths S, Stuhler G, et al. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. Blood 2000;96:3102-8.
52. Morse MA, Deng Y, Coleman D, et al. A phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen. Clin Cancer Res 1999;5:1331-8.
53. Thurner B, Roder C, Dieckmann D, et al. Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. J Immunol Methods 1999;223:1-15.
54. Rouard H, Leon A, Klonjkowski B, et al. Adenoviral transduction of human “clinical grade” immature dendritic cells enhances costimulatory molecule expression and T-cell stimulatory capacity. J Immunol Methods 2000;241:69-81.
55. Coulon V, Ravaud A, Huet S, et al. In vitro production of human antigen presenting cells issued from bone marrow of patients with cancer. Hematol Cell Ther 1997;39:237-44.
56. Maffei A, Ayello J, Skerrett D, et al. A novel closed system utilizing styrene copolymer bead adherence for the production of human dendritic cells. Transfusion 2000;40:1419-20.
57. Harris P, Hesdorffer C. Growth of human dendritic cells for cancer immunotherapy in closed system using microcarrier beads. Google Patents; 2002.
58. Bernard J, Ittelet D, Christoph A, et al. Adherent-free generation of functional dendritic cells from purified blood monocytes in view of potential clinical use. Hematol Cell Ther 1998;40:17-26.
59. Cao H, Verge V, Baron C, et al. In vitro generation of dendritic cells from human blood monocytes in experimental conditions compatible for in vivo cell therapy. J Hematother Stem Cell Res 2000;9:183-94.
60. Elias M, van Zanten J, Hospers GA, et al. Closed system generation of dendritic cells from a single blood volume for clinical application in immunotherapy. J Clin Apher 2005;20:197-207.
61. Eyrich M, Schreiber SC, Rachor J, et al. Development and validation of a fully GMP-compliant production process of autologous, tumor-lysate-pulsed dendritic cells. Cytotherapy 2014;16:946-64.
62. Garderet L, Cao H, Salamero J, et al. In vitro production of dendritic cells from human blood monocytes for therapeutic use. J Hematother Stem Cell Res 2001;10:553-67.
63. Gullen D, Abe F, Maas S, et al. Closing the manufacturing process of dendritic cell vaccines transduced with adenovirus vectors. Int Immunopharmacol 2008;8:1728-36.
64. Mu LJ, Gaudernack G, Saebø-Larssen S, et al. A protocol for generation of clinical grade mRNA-transfected mono-ocyte-derived dendritic cells for cancer vaccines. Scand J Immunol 2003;58:578-86.
65. Garritsen HS, Macke L, Meyring W, et al. Efficient generation of clinical-grade genetically modified dendritic cells for presentation of multiple tumor-associated proteins. Transfusion 2010;50:831-42.
66. Macke L, Garritsen HS, Meyring W, et al. Evaluating maturation and genetic modification of human dendritic cells in a new polyolefin cell culture bag system. Transfusion 2010;50:843-55.
67. Wong EC, Lee SM, Hines K, et al. Development of a closed-system process for clinical-scale generation of DCs: evaluation of two monocyte-enrichment methods and two culture containers. Cytotherapy 2002;4:65-76.
68. Erdmann M, Dorrie J, Schaft N, et al. Effective clinical-scale production of dendritic cell vaccines by monocyte elutriation directly in medium, subsequent culture in bags and final antigen loading using peptides or RNA transfection. J Immunother 2007;30:663-74.
69. Tan YF, Sim GC, Habshah A, et al. Experimental production of clinical-grade dendritic cell vaccine for acute myeloid leukemia. Malays J Pathol 2008;30:73-9.
70. Kurlander RJ, Tawab A, Fan Y, et al. A functional comparison of mature human dendritic cells prepared in fluorinated ethylene-propylene bags or polystyrene flasks. Transfusion 2006;46:1494-504.
71. Guýre CA, Fisher JL, Waugh MG, et al. Advantages of hydrophobic culture bags over flasks for the generation of monocyte-derived dendritic cells for clinical applications. J Immunol Methods 2002;262:85-94.
72. Suen Y, Lee SM, Aono F, et al. Comparison of monocyte enrichment by immuno-magnetic depletion or adherence for the clinical-scale generation of DC. Cytotherapy 2001;3:365-75.
73. Laus R, Vidovic D, Graddis T. Compositions and methods for dendritic cell-based immunotherapy. Google Patents; 2006.
74. Curtis AS, Forrester JV, McInnes C, et al. Adhesion of cells to polystyrene surfaces. J Cell Biol 1983;97:1500-6.
75. Ryan JA. Evolution of cell culture surfaces. BioFiles 2008;3:8-11.
76. Curtis ASG, Forrester JV, Clark P. Substrate hydroxylation and cell adhesion. J Cell Biol 1986;106:924-5.
77. Loh JH. Plasma surface modification in biomedical applications. Med Dev Technol 1999;10:24-30.
78. Battiston KG, McBane JE, Labow RS, et al. Differences in protein binding and cytokine release from monocytes on commercially sourced tissue culture polystyrene. Acta Biomater 2012;8:89-98.
79. Steele JG, Dalton BA, Johnson G, et al. Adsorption of fibroblasts. Biomaterials 1995;16:1057-67.
FEKETE ET AL.

82. Yohko FY. Kinetics of protein unfolding at interfaces. J Phys Condens Matter 2012;24:503101.
83. Library TF. Nexell therapeutics launches next generation cell culture containers. Business Wire; 2000. Available from http://www.thefreelibrary.com/Nexell+Therapeutics+Launches+Next+Generation+Cell+Culture+Containers.-a059581372.
84. Motta MR, Castellani S, Rizzi S, et al. Generation of dendritic cells from CD14+ monocytes positively selected by immunomagnetic adsorption for multiple myeloma patients enrolled in a clinical trial of anti-idiotypic vaccination. Br J Haematol 2003;121:240-50.
85. Heal JM, Brightman A. Cryopreservation of hematopoietic progenitor cells collected by hemapheresis. Transfusion 1987;27:19-22.
86. Strasser EF, Keller B, Hendelmeier M, et al. Short-term liquid storage of CD14+ monocytes, CD11c+, and CD123+ precursor dendritic cells produced by leukocytapheresis. Transfusion 2007;47:1241-9.
87. Gangal SV. Fluorine-containing polymers, perfluorinated ethylene-propylene copolymers. In: Kirk-Othmer encyclopedia of chemical technology. Hoboken (NJ): John Wiley & Sons; 2000.
88. Plunkett R. The history of polytetrafluoroethylene: discovery and development. In: Seymour R, Kirshenbaum G, editors. High performance polymers: their origin and development. Utrecht: Springer Netherlands; 1986:261-6.
89. Gangal SV, Brothers PD. Perfluorinated ethylene-propylene copolymers. In: Encyclopedia of polymer science and technology. Hoboken (NJ): John Wiley & Sons; 2002.
90. Teng H. Overview of the development of the fluoropolymer industry. Appl Sci 2012;2:496.
91. Saint-Gobain Performance Plastics. LabPure® FEP Bags. Saint-Gobain Performance Plastics; 2015.
92. Warner BD, Dunne J. Fluid exchange methods and devices. Google Patents; 2013.
93. Figdor CG, de Vries IJ, Lesterhuis WJ, et al. Dendritic cell immunotherapy: mapping the way. Nat Med 2004;10:475-80.
94. Kaiser AD, Assenmacher M, Schroder B, et al. Towards a commercial process for the manufacture of genetically modified T cells for therapy. Cancer Gene Ther 2015;22:72-8.
95. Fekete N, Rojewski MT, Furst D, et al. GMP-compliant isolation and large-scale expansion of bone marrow-derived MSC. PLoS One 2012;7:e3255
96. Lamers C, Guest R. T-cell engineering and expansion—GMP issues. In: Hawkins RE, editor. Cellular therapy of cancer: development of gene therapy based approaches. Singapore: World Scientific Publishing; 2014. p. 131-77.
97. Wilson JR, Page DA, Welch D, Robeck A. Cell culture methods and devices utilizing gas permeable materials. Google Patents; 2012.
98. Levine DW, Wang DIC, Thilly WG. Optimizing parameters for growth of anchorage-dependent mammalian cells in microcarrier culture. In: Acton RT, Lynn JD, editors. Cell culture and its application. San Diego: Academic Press; 1977. p. 191-216.
99. Munder PG, Modolell M, Hoelzl Wallach DE. Cell propagation on films of polymeric fluorocarbon as a means to regulate pericellular pH and pO2 in cultured monolayers. FEBS Lett 1971;15:191-6.
100. Jensen MD. Production of anchorage-dependent cells—problems and their possible solutions. Biotechnol Bioeng 1981;23:2703-16.
101. House W, Shearer M, Maroudas NG. Method for bulk culture of animal cells on plastic film. Exp Cell Res 1972;71:293-6.
102. Wilson JR, Page DA, Welch DP, et al. Cell culture methods and devices utilizing gas permeable materials. Google Patents; 2014.
103. Hauser AHR. Process development and manufacturing. Singapore: World Scientific Publishing; 2016.
104. McKeen LW, Massey LK. Permeability properties of plastics and elastomers. 3rd ed. Amsterdam, Boston: Elsevier; 2012.
105. Nass LI, Heiberg CA. Encyclopedia of PVC. 2nd ed. New York: Marcel Dekker; 1986.
106. Patrick S. Practical guide to polyvinyl chloride. Shrewsbury, UK: Rapra Technology; 2005.
107. Hammond M, Nunn H, Rogers G, et al. Identification of a leachable compound detrimental to cell growth in single-use bioprocess containers. PDA J Pharm Sci Technol 2013;67:123-34.
108. Pahl I, Dorey S, Barbaroux M, et al. Analysis and evaluation of single-use bag extractables for validation in biopharmaceutical applications. PDA J Pharm Sci Technol 2014;68:456-71.
109. Thiele J, Ma Y, Bruekers SM, et al. 25th anniversary article: Designer hydrogels for cell cultures: a materials selection guide. Adv Mater 2014;26:125-47.
110. Gilbert PM, Havenstrite KL, Magnusson KE, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 2010;329:1078-81.
111. Swift J, Ivanovska IL, Buxboim A, et al. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 2013;341:1078-81.
112. Yang C, Tibbitt MW, Basta L, et al. Mechanical memory and dosing influence stem cell fate. Nat Mater 2014;13:645-52.
113. Eyckmans J, de Vries IJ, Luscher TF, et al. Mechanical memory: a materials selection guide. Adv Mater 2014;26:125-47.
114. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech 2011;4:165-78.
115. Saint-Gobain. Frequently asked questions. 2012-2014. Available from http://americanfluoroseal.com/faqs
116. Buñi N, Saitakis M, Dogniaux S, et al. Human primary immune cells exhibit distinct mechanical properties that are modified by inflammation. Biophysical Journal 2015;108:2181-90.
117. Elkin BS, Azeloglu EU, Costa KD, et al. Mechanical heterogeneity of the rat hippocampus measured by atomic force microscope indentation. J Neurotrauma 2007; 24:812-22.

118. Miyake K, Satomi N, Sasaki S. Elastic modulus of polystyrene film from near surface to bulk measured by nanoindentation using atomic force microscopy. Appl Phys Lett 2006;89:

119. Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. Nat Mater 2014;13:547-57.

120. The Chemours Company FC, LLC. Teflon™ FEP fluoroplastic film properties bulletin. The Chemours Company FC, LLC; 2014.

121. Vogler EA. Protein adsorption in three dimensions. Biomaterials 2012;33:1201-37.

122. Parhi P, Golas A, Vogler EA. Role of proteins and water in the initial attachment of mammalian cells to biomedical surfaces: a review. J Adhes Sci Technol 2010;24:853-88.

123. Garcia-Nieto S, Johal RK, Shakesheff KM, et al. Laminin and fibronectin treatment leads to generation of dendritic cells with superior endocytic capacity. PLoS One 2010;5: e10123.

124. Poudel B, Yoon DS, Lee JH, et al. Collagen I enhances functional activities of human monocyte-derived dendritic cells via discoidin domain receptor 2. Cell Immunol 2012;278: 95-102.

125. Sidaway P. Immunotherapy: sipuleucel-T induces humoral antigen spread in patients with mCRPC. Nat Rev Urol 2015; 12:181

126. Sabado RL, Bhardwaj N. Cancer immunotherapy: dendritic-cell vaccines on the move. Nature 2015;519:300-1.

127. Ahmed MS, Bae YS. Dendritic cell-based therapeutic cancer vaccines: past, present and future. Clin Exp Vaccine Res 2014;3:113-6.

128. Obermaier B, Dauer M, Herten J, et al. Development of a new protocol for 2-day generation of mature dendritic cells from human monocytes. Biol Proced Online 2003;5:197-203.

129. Fox BA, Schendel DJ, Butterfield LH, et al. Defining the critical hurdles in cancer immunotherapy. J Transl Med 2011;9: 214.