CELL & GENE THERAPY INSIGHTS

OVERCOMING DOWNSTREAM BOTTLENECKS IN CELL & GENE THERAPY MANUFACTURING

SPOTLIGHT

EXPERT INSIGHT

Bioprocess engineering strategies for autologous human MSC-based therapies: one size does not fit all

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Autologous cell therapies are currently being evaluated in multiple clinical trials and are becoming a reality in advanced healthcare services. Compared to allogeneic cell therapies, where one batch of cells can be used to treat multiple patients and which allows a business model that is more closely related to the traditional biologics, autologous or patient-specific cell-based therapies present a whole new set of challenges. While these new challenges can originate from ethical issues (e.g., concerns about the patient in cases of batch failure) or from safety concerns (e.g., cross-contamination between patients), this article provides an overview of the technical side of cell-therapy manufacturing that is subject to donor-related variability. Although several studies have managed to produce batches of cells with a scale that satisfies therapeutic needs, there are still a number of challenges that need to be tackled. Unlike traditional manufacturing processes where the input material is relatively constant over time, personalization aspects inherent to the autologous reality will expose manufacturing to significant variability and production risks. The authors argue that for autologous cell production, where every patient-specific production batch can be considered as an unknown process, a combination of automated production processes and robust process monitoring & control capabilities can provide quantitative process understanding. At a second stage, provided large data becomes available through (on-line) data-based process analytics, minimized risk and cost-effective cell production for clinical use will become a reality.

Submitted for review: May 19 2017 ▶ **Published**: Aug 3 2017

Autologous cell therapy focuses on bioprocesses that allow a patient's own cells to be manufactured into an advanced therapy medicinal product (ATMP). The major advantage of autologous cell therapies is that the implanted cells will not trigger a graft-vs-host immune response thus reducing the need for immunosuppression. Therefore in autologous therapy, production batches are expected to be patient-specific

whereby the ATMP is used only for that patient. Compared to allogeneic treatments, where a production batch serves multiple patients, the autologous manufacturing strategy inherently possesses specific challenges that are attributed to limited economies of scale, risks associated to batch failure, bioprocess flexibility and production planning. A subdivision of autologous ATMPs are mesenchymal stromal cell (MSCs)-based products. More than 400 clinical trials use currently human mesenchymal stromal cells as a therapeutic cell source [1,2], the growth and potential benefits that can be brought by cell-based therapy seems to be recognized by industry. Consequently, more and more cell-based companies arise leading in a substantial growth of the field which moves towards a distinct sector of the healthcare industry [3,4]. Currently, a challenge that has been faced by the emerging ATMP industry is the preparation of cell -based ATMPs under "hospital expemption" as an alternative regulatory pathway towards the patient. Also in combination with high costs required for GMP manufacturing and maintenance, hospital exemption has been linked to the the very low percentage of translation from academic development of ATMPs towards regular clinical care [5]. Through this specific regulatory pathway ATMPs can reach patients earlier and without having to go through the cumbersome process of getting the cell product to commercial scale [6]. An additional complication in the European Union is the existence of big regulatory differences among member states regarding hospital expemtpion and GMP manufacturing in hospital settings [7]. The alliance for advanced therapies (AAT) has emphasized that the inconsistent implementation of the hospital exemption in the EU member states and routine preparations of treatments under an exemption impede the development of new safe and cost-effective treatments [8].

It is expected that most autologous cell therapy applications batches should be able to deliver cell numbes ranging between 10^6 and 10^9 cells for a single dose [9,10]. The adoption of monitored and controlled bioprocesses that are able to guarantee the production of cell-based therapies with manageable cost of goods (COGs) and robust *in vivo* performance [11–14] has been identified as an important prerequisite to address the currently low number of Phase III clinical trials translated into viable commercial products. Upstream challenges have been to date addressed in terms of the quantities of cells that need to be produced. Bioreactors have been increasingly tested in large volumes and used for ATMP production due to the fact that they provide an improved cell culture environment by controlling nutrient refreshment and waste removal rates [15], flexibility in scale of operation, while reducing bioprocess complexities [16]. The use of bioreactor systems with various designs for the expansion of human MSCs is a reality and is by now adopted for industrial production, reflecting that major concerns regarding large-scale MSC production are addressed. This is indicated by an increasing number of recent review papers [17–19] including successes in MSC expansion in academic, hospital and industrial settings [20]. However, extensive investigation of downstream processes such as centrifugation and filtration and vialing has only recently been carried out.

DOI: 10.18609/cgti.2017.040

SOURCES OF VARIATION FOR MSC-BASED BIOPROCESSING

Existing literature and regulatory perspectives [21] discuss a very broad number of parameters that should be taken into account when designing bioprocesses for the expansion of MSC populations. These factors should be classified as sources of variability and could all affect bioprocess efficiency and result. Below is a list and breakdown of possible sources of variation during MSC-based manufacturing:

Donor profile is a major source of variability in autologous MSC therapy since the donor to donor difference can be linked not only to the genetic profile of each patient but also to factors such as disease and life style [22]. In addition, donor-related variability can affect not only the final product characteristics, but also in-process performance and sensitivity to bioreactor operating parameters. Accordingly, process screening and optimization experiments should include basic characterization of donor sensitivity to process parameters [22].

Most commonly used cell types are bone marrow [23], umbilical cord [24], adipose [25], synovium [26] and periost [27]. An autologous specific challenge is that MSC numbers harvested from biopsies differs because of the way biopsies are obtained by clinicians. In addition, tissue specific biopsies contain different fractions and numbers of true progenitor cells. For example 1 mL of human MSCs from the bone marrow results approximately in 10^3 MSCs [28]. On the other hand, adipose-derived MSCs count for 0.5–2.0 × 10^6 cells/g of adipose tissue. From these cell numbers the percentages of MSCs range from 1 to 10% [29]. As illustrated there is not only an inherent interdonor variability but also an intradonor variability in the basic "raw material" of the autologous paradigm.

A large range of culture setups used to support MSC expansion contributes to donor specific bioprocess efficiency and variability. For instance microcarrier-based stirred tank reactors [30], hollow fiber [31], wave bags [32] and multiplate bioreactors [33] have been successfully employed to generate large-scale batches of MSCs for autologous applications. In addition, donor responses to culture plastic, and by extension any culture surface in general, itself can potentially induce variability in cell yields [34].

There is a large variety of customized media formulations for cell expansion, entailing for example different protein sources or glucose concentrations. Media containing non-defined sources of protein such as Fetal Bovine Serum (FBS) and Human Platelet Lysate (HPL) expose ATMP manufacturing to batch-dependent process variability. Moreover they pose a considerable risk for pathogen transmission. Due to this, great effort is focused in the development of chemically defined xeno-free media that would allow efficient and controlled MSC growth [35,36]. An overview of serum-free media available for MSC expansion can be found in [37]. Finally, cryopreservation media used to freeze products (in cases where transport to the patient is required) could also affect in a different way cells from different donors.

Bioprocess operating conditions, for instance cell seeding densities per passage, media refreshment strategies, response to shear type and magnitude, perfusion rates and

dissolved oxygen tension, vary for almost every process [38] and could affect process performance in a donor related context [39].

The aforementioned parameters reveal that autologous MSC expansion and product development is an extremely complex and challenging bioprocessing endeavour. In addition, constantly changing metrics for bioprocess characterization have been to date employed to quantify bioprocess efficiency. This results in incomparability between investigations based on the reported results requiring substantial data postoprocessing. For instance the calculation of "population doubling time" and "fold increase" obtained as a metric after a cell expansion process differs between report, sometimes starting from a theoretical seeding density or an estimate of the successfully seeded/attached number of cells in a bioreactors system [36].

We would like to stress the need for critical and systematic analysis of existing information that could result in: (i) process comparability (ii) process benchmarking (iii) improved understanding of bioprocess efficiency (iv) risk assessment, all resulting in rational design of whole autologous bioprocesses.

ON QUALITY CONTROL & PRODUCT COMPARABILITY

A major challenge that needs to be addressed by cell-based ATMP manufacturers is to demonstrate and maintain product comparability after changes have been introduced in the bioprocess [40]. This is expected to occur as bioprocesses involved in product manufacturing are optimized or altered. This is expected for instance to occur when moving from a 2D to a 3D platform for MSC culture or when automation is introduced in the manufacturing line [41]. Therefore, assays ensuring product quality and allowing proof of product comparability, establishing product safety and efficacy, are required. However, in the field there is increasing concern even on the use of the generic term "Mesenchymal Stromal Cells (MSCs)" which has been used as an umbrella term encompassing multiple progenitor populations derived from a variety of sources [42]. To characterize these cells, to date, a minimal set of criteria where suggested from the International Society of Cell Therapy (ISCT) comprising of adhesion to plastic, several CD markers and in vitro differentiation tests [43] and have since been extensively adopted as proof of multipotency of an "MSC" population. However we should emphasize that currently, no single-cell surface marker is available for the unambiguous identification of MSCs. In addition these metrics do not reflect the identity or potency of MSC populations. A recent report revealed that committed progenitors of distinct origin (that would all be characterized as MSCs) where characterized by *in vivo* bone forming assays [44]. Moreover, pericytes from different sources (cell types under the generic term MSCs) seem to exhibit remarkably different behavior and differentiation potential [45].

Therefore the discovery of quality controls of higher biological specificity and discriminative power that would also link to cell potency are urgently needed. Ideally these metrics should be also linked to the mechanism of action of the

DOI: 10.18609/cgti.2017.040

harvested and expanded progenitor cell populations. New technologies for epigenetic analyses of cells could provide important information regarding the classification of types [46] based on their tissue of origin even after extensive culture, and about bioprocessing during differentiation of MSCs and its impact on epigenetic patterns [47]. The generation of specific epigenetic signatures reflects functional properties of MSCs such as their hematopoiesis supportive function [48]. Epigenetic signatures could be also developed in order to evaluate the therapeutic potential of MSCs after validation with suitable datasets.

Variation in starting materials is a significant challenge and the ability within the field to quantify and potentially control it will require the adoption of systematic and quantitative metrics as already mentioned. Potential ways of defining bioprocessisng metrics could be carried out and even the adoption of reference MSC lines has been suggested as a calibration tool [49,50]. In addition, knowledge on the impact of manufacturing at different sites on MSC product quality is still rather limited and should be explored as this model of production would rely on such production and distribution strategies. In a recent study eight centers carried out manufacturing of BMSCs for early phase clinical trials following Good Manufacturing Practice (GMP). They observed substantial differences between locations in both the *in vitro* properties of the cells as well as their performance *in vivo* in terms of bone formation and bone marrow formation on ceramic carriers exhibiting low comparability across sites [51].

RISK ASSESSMENT & MANAGEMENT

The application of risk assessment has been mostly applied to automated processes, as for instance the ones encountered in the biopharma industry. However in the case of the autologous MSC based production, which is to date highly dependent on manual intervention, this poses considerable challenges. A number of such tools have been employed such as risk ranking, hazard analysis and critical control points (HACCP), hazard operability analysis and failure mode and effects analysis (FMEA) which allow highlighting of potential errors and define actions to perform to prevent consequences. In addition, more quantitative approaches, such as the failure modes effects and criticality analysis (FMECA) that quantify errors, could be also implemented in the field for managing these risks when moving to translational setting. Risk assessment for autologous processes becomes more complex due to for instance donor variability, e.g. the donor-specific population doubling times require higher flexibility for production planning, multiple sources of donor material might increase the chance of contamination, donor-related lot-to-lot variability complicates the use of classical statistical process control techniques (e.g., control charts and process capability analysis [52]. Stochastic modelling techniques such as Monte Carlo simulations and risk-based analysis could help to gain more insight on the impact of these sources of variation on the production process [53].

Only a handful of studies demonstrate the adoption of risk management tools for cell therapy

manufacturing and these examples comprise of liver progenitor and chondrocyte cells. An example of adoption of a failure mode and effects analysis (FMEA) can be seen in Lopez *et al* [54] whereby risks were identified and prioritized and a severity/occurrence matrix was highlighted for the production of liver progenitor cells. In another study it was shown that the implementation of an FMEA/FMECA method revealed the causality of human errors (either due to errors or inadequate training) and the subsequent introduction of 26 criticalities within GMP production of autologous chondrocyte implantation [55]. Currently, a major challenge in the field for the implementation of similar approaches is the absence of reference standards for determining acceptable risk levels and this should be further explored.

Since the field is currently expanding both in terms of MSC-based products but also in terms of novel automated bioprocesses supporting cell expansion (multiple bioreactor types), cell separation, condensation and fill and finish and freezing steps, similar strategies could be followed during their integration and adoption in the production pipeline. Risk assessment for the determination of critical quality attributes of the MSC-based product should be carried out for each bioprocess step. Similarly the classification of the most influential process parameters of each bioprocess on its performance as well as that of material attributes (for the MSC case donor related and also niche/origin related) should be also carried out. Currently there is limited literature on this topic for MSC based bioprocesses.

Considering cost-effectiveness during bioprocess design

The integration of process performance with cost estimates requires the maintenance of several culture parameters across culture systems. As this is rather a challenging endeavour only limited information is currently available. For an autologous example this was carried out for multiple standardized large-scale expansion processes and reported recently by Lambrechts *et al* [56]. The calculation compared the expansion of 20 million MSC-like cells (human periosteum derived progenitor cells] to 350 million cells in high-glucose DMEM supplemented with 10% irradiated FBS in T175 tissue culture flasks, a hollow fiber bioreactor (Terumo BCT Quantum® Cell Expansion System, [57]) a multiplate bioreactor (Pall Integrity Xpansion, [33]) and in a spinner flask with CultiSphere-S microcarriers (unpublished results). The general conclusion here was that for this relatively smaller scale (autologous case study) the microcarrier-based expansion process is most likely to result in the lowest production costs, while the ease of use of the hollow fibre and multi-plate bioreactor is offset by a high cost of the disposable materials. However this was based on current values and expenses that could gradually change and adapt to a growing cell therapy market.

Similar results can be found for large-scale (allogeneic) expansion processes. For example, the economics of allogeneic expansion for pluripotent stem cells (iPSCs and ESCs) have been recently modeled and described both for the upstream [58] as well as for the subsequent downstream operations [59].

DOI: 10.18609/cgti.2017.040

This is not yet clearly addressed in the case of autologous large-scale expansion, where most likely a generic solution does not exist and optimal solutions will be case specific, based on inherent cell or donor properties as well as practical limitations (e.g. cells obtained from biopsies vs. cells required for therapy) [12]. Additionally, the cost of quality control requires more specific attention in these process models, since the additional cost per dose is weighing more on the autologous case because of the lack of economies of scale. Due to the inherent donor-related variability and uncertainty involved in these processes, the use of such deterministic models for the autologous case would therefore be a challenging task.

SCALABILITY OF MSCS

The discussion regarding scalability for autologous and allogeneic cell production on the basis of "scale-out" or "scale-up" has by today clarified best practice for each case. While scale-out is simply carrying out numerous same and "smaller" scale bioprocesses (parallelisation of processes with the same dimensions), scale-up envisions a direct increase in volume or surface of the culture. For autologous expansion processes, the production strategy of choice is not yet carved in stone. The available bioreactor culture scale must take into account the flexibility to accommodate the range of cell growth across all batches and also the fact that doses might also differ based on patient profile, i.e. personalized dosing. A number of systems exist in the market offering GMP-grade such as the hollow fibre system by Terumo (Quantum) and the Xpansion series by Pall both allowing to reach batches of $5x10^8$ cells suitable for multiple autologous applications. A bottleneck in the adoption of these systems is the high number of cells needed for seeding $10\text{-}20x10^6$ cells which is much higher than the cell number obtained from biopsies. Therefore a 2D culture step would be needed to reach the required number of MSCs for bioreactor seeding. In addition, suspension bioreactors (i.e., Xuri and PBS biotech) with microcarriers allow a high degree of flexibility in terms of scale, crucial for the autologous case study.

In addition we would like to also stress the cost efficiency of bioreactor systems especially when the manufacturing footprint comes into the picture allowing for more compact platforms for product delivery requiring less personnel to carry out manufacturing [60]. The use of monitored and controlled bioreactors allows process automation (e.g. automated liquid transfer steps), which in turn achieves a reduction in labor requirements as well as improvements in quality assurance. Together, these steps serve not only to reduce the cost for patient specific manufacturing, but also to allow scale-out strategies whereby multiple batches are simultaneously manufactured, potentially in multiple non-centralized facilities. Further, the increased control afforded by bioreactors serves to reduce the risk of batch failures through early fault detection, efficiently using scarce donor materials and reducing the time patients wait for a therapy. Additionally, the more individualized process of which the production is often aimed to take place close to the bedside of the patient (distributed manufacturing) seems

to require more integrated systems, where cell expansion, volume reduction and cell sorting are enclosed in one automated device (e.g. the Octane Cocoon bioreactor and Miltenyi Biotec's Prodigy). These versatile and low footprint compact devices could also be adopted for point of care manufacturing within hospital facilities, which could be a second strategy for manufacturing autologous MSC-based ATMPs in contrast to a more centralized manufacturing model [60].

DOWNSTREAM BIO-PROCESSES & PROCESS INTEGRATION

The production of MSCs for autologous therapy and especially the "upstream" component of the whole-bioprocess has been investigated thoroughly, dealing with scalability and GMP aspects. However, in order to consider whole-bioprocess design there needs to be equal attention and effort on the development of efficient and flexible downstream bioprocesses. More particularly, the new generation of downstream processes should be able to address the increasing volumes and batches of cells produced at the upstream stages, but also possess flexibility in operation for supporting numerous autologous expansion processes. For instance, MSC harvest from microcarriers in suspension bioreactors was recently investigated providing a scalable methodology [61], while in a follow up study the authors linked this process to the subsequent cryopreservation step [36,61]. Dynamic harvest of single cells from fixed bed bioreactors was also recently described for the recovery of periost derived progenitor cells which retained their bone froming potential in vivo [62]. Downstream operations are becoming of significant importance for stem cell bioprocessing, and initial theoretical discussions on separation techniques possibly useful for the cell therapy field as reviewed by Diogo *et al.* [63] are becoming a reality. There is a growing rate of studies on bioprocesses and methods required for the clarification and volume reduction of MSC suspensions using membranes and tangential [64,65] or dead-end [66] filtration. Established techniques in other biotechnological fields are also successfully adopted such as the use of expanded bed chromatography for the washing of MSC suspension resulted in improved efficiency [67]. This shows the rapid evolution of the field in reaching a pipeline of unit operations for (autologous) MSC manufacturing, customized per application and from patient-to-patient.

Making use of scale-down autologous bioprocess pipelines

Given the high-risk associated with autologous bioprocesses, scale down methodologies are needed for quantifying and exploring the complexities of personalized bioproduction. The evaluation of potentially optimal windows of bioprocess operation could suggest similar culture conditions for larger scale formats provided proper scaling parameters are used. Although commercially systems are available for suspension culture, the volumes used are still in the order of magnitude of 10s of millilitres. We believe that there is room for pursuing the development of mini bioreactors that allow a further scale-down, while in parallel increase throughput.

DOI: 10.18609/cgti.2017.040

Reichen *et al* [68] have shown that the quantification of cell specific attributes can be measured in high throughput microfluidic devices, however the behaviour of cells on flat surfaces may not be necessarily predictive of that on surfaces possessing curvature such as for instance on microcarriers. Shear stress is a factor that can be encountered in multiple units of operation from impeller shear to acute capillary shear during flow through orifices and capillaries. The impact of such shear magnitudes on cells destined for cell therapy has been carried out in small (μL) volumes [69,70] showing that fluid flow conditions could be fine-tuned to minimizing cell loss and cell property degeneration. Scale down strategies aiming to mimic the effects of specific downstream processes were also recently shown by Delahaye *et al* [71] for dead-end centrifugation, and by Masri *et al* [72] for a membrane separation and recovery bioprocess. We could therefore suggest that such scale down tools could provide a first solid basis for evaluating optimal bioprocess integration scenario at a low cost and low risk environment. Moreover the production of low-cost high-throughput time series data sets could also be achieved helping considerably in uncertainty quantification capturing potentially similar trends with the large scale units of operation.

RATIONAL DATA-BASED BIOPROCESS DESIGN

A growing discussion on standardization, given the large variety of complex components involved in cell therapy research and development [73], highlights that its absence is an obstacle for the transition of autologous cell-based therapeutics from the development phase that is often based on trial and error, to the translational stage that requires robust and cost-effective processes [74]. For instance, the adoption of improved standards for cell-based ATMPs entering clinic [75] was recently suggested. Regarding input cell material, recent literature has highlighted the need for standardized MSC lines as calibration tool [49] or reference material [76] while a systematic data-based approach and centralized manufacturing facilities able to conduct systematic comparability studies was advocated by McKenna *et al.* [77], highlighting the invasiveness of the immortalization step to the initial cell properties. Moreover, in order to address the complex regulatory landscape, a cell therapy regulatory toolkit (online regulatory resource) was introduced for new ATMPs entering clinical trials for the EU and USA [78]. It is clear that a certain degree of standardization would be helpful to move the field forward, however many cell-based therapies (in particular autologous therapies) will require personalized approaches where flexibility is required in order to allow customization per patient or per therapy.

FUTURE CHALLENGES

The lack of quantitative understanding of a series of factors that would enable rational bioprocess design and risk minimization is still a major threat in establishing viable production lines. The call for inclusion of process analytical technology (PAT) tool in cell therapy biomanufacturing is much more

crucial in the autologous case. We believe that the incorporation of data based tools, that are able to treat time-series data from diverse bioprocesses and automatically providing quantitative metrics for each bioprocess, could be a first considerable step to personalized autologous biomanufacturing pipelines. Time series of process parameters that quantitatively describe the dynamic culture environment [79] could provide much more insightful information on the dynamics as well as the robustness of autologous bioprocesses.

While for most process control strategies on-line measurements of specific Critical Quality Attributes (CQAs) are required, this is not always feasible in the autologous case. From a practical point of view, the limited donor material does not allow for frequent (destructive) sample taking during culture, while the comparability of very specific CQA measurements (e.g. by mass spectrometry or ELISA) is challenging in the often distributed manufacturing facilities. From a cost perspective, an expensive CQA measurement is much more detrimental for the cost-effectiveness of an autologous strategy compared to an allogeneic strategy where this cost can be distributed over multiple patients. Model-based monitoring and control strategies, where a combination of multiple (easy to measure) process parameters such as metabolite consumption and oxygen uptake are combined with a predictive model that allows to estimate the state of a CQA are therefore a promising strategy for the cost-effective production of autologous therapies [80].

Future challenges will also lie on the identification of more specific CQAs. Although the ISCT criteria provide minimum cell identification criteria [43], it would be crucial for autologous processes to be linked to potency assays. This will be a major challenge as increased sensitivity and functionality CQAs would be cell type and application specific. Currently there is minimal information in literature describing and linking the performance of expanded autologous cells in *in vivo* settings. This info, which is usually omitted and only seldom linked to its bioprocess history, would prove that expanded cells where actually potent and useful for clinical application. The need for such predictive potency assays, linking the process *in vivo* activity and defining actually the critical process parameters, would also ensure product comparability during subsequent manufacturing phases [81].

CONCLUSION

The steady increase in MSC production scales demonstrates the continuous maturation of the field. However, mainly for autologous therapies, there are considerable bioprocess engineering challenges to be faced for the successful transition from early preclinical to late commercial stage manufacturing. A major factor contributing to this challenge is the donor-related variability in combination with the fact that there is no typical, one-size-fits-all manufacturing solution. Metrics allowing the identification of MSC progenitor subpopualtions and added markers predicting potency are necessary for comparability and technology transfer endeavours. Further understanding basic biology and mechanism of action of

DOI: 10.18609/cgti.2017.040

MSC-based therapeutics and the implementation of novel technologies for quantifying this is also needed. The implementation of risk assessment and management strategies will also allow translating the field to a mitigated manufacturing field ready to address current clinical needs. The development of product and process specific data-signatures derived from the incorporation of sensors in all bioprocess steps, could help in increasing our understanding of dealing with personalized biomanufacturing.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

TL is supported by a Flanders Innovation & Entrepreneurship (VLAIO) Innovation Mandate (HBC.2016.0629). IP is funded by Fonds Wetenschappelijk Onderzoek (FWO fellowship, project No 12O7916N). Part of the research is supported by the KU Leuven Concerted Research Actions (GOA/13/016). The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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DOI: 10.18609/cgti.2017.040