Physical Chemistry & Biophysics

Research Article Open Access

Novel Automated Patch-clamp Assays on Stem Cell-derived Cardiomyocytes: Will they Standardize *In Vitro* Pharmacology and Arrhythmia Research?

Amuzescu B*, Scheel O and Knott T

Cytocentrics Bioscience GmbH, Rostock, Germany

Abstract

Recent progress in embryonic stem cell and human induced pluripotent stem cell technology allowed effective generation of cultured cardiomyocyte preparations with over 99% purity, rendering them suitable for automated patch-clamp approaches. Compared to current high-throughput drug screening methods, such as fluorescence assays using calcium-sensitive or transmembrane potential-sensitive dyes, or field potential recordings and activation mapping using multi-electrode arrays, patch-clamp experiments offer the possibility to combine action potential recordings in current-clamp mode with detailed characterization of drug effects on multiple ion current components with carefully designed voltage-clamp protocols, leading to an in-depth understanding of arrhythmogenesis conditions and mechanisms, especially when combined with cellular electrophysiology computerized models. The recently issued Comprehensive *in vitro* ProArrhythmia Assay (CiPA) guidelines emphasize the importance of pharmacological tests on multiple cardiac ion channels, including at least Nav1.5 (early and late), Cav1.2, hERG1, Kv7.1/minK, and Kir2.1, via voltage-clamp protocols, instead of simple hERG screening, combined with computer modeling, in order to determine the proarrhythmic liability of a drug candidate. In addition, patch-clamp assays on patient-specific induced pluripotent stem cell-derived cardiomyocytes will enhance current molecular diagnosis methods in cardiac channelopathies by identification of the faulty current component and individualized screening of drug sensitivity of mutant channels, a step forward for personalized medicine approaches.

Keywords: Automated patch-clamp; Cardiac cell electrophysiology model; Cardiac channelopathy; Drug safety high-throughput screening; Cipa; MICE; Human embryonic stem cell-derived cardiomyocyte; Human induced pluripotent stem cell-derived cardiomyocyte; Patient-specific induced pluripotent stem cell-derived cardiomyocyte; Calcium-sensitive dye; Transmembrane voltage-sensitive dye; Multielectrode array; Field potential; Action potential

Introduction

The first significant achievements in cellular cardiac electrophysiology date back to the beginning of the 1950s, when the two-microelectrode technique was applied to a variety of preparations, most notably "false tendons" of dog, kid, or lamb heart [1]. The Purkinje cells contained in these tissue samples present several important features, including a larger diameter (40-100 μm) and length and a lower contractility compared to ordinary ventricular fibres, enhancing multiple impalement and long stable recordings (over 10 minutes), an almost complete insensitivity to parasympathomimetic agents, and higher densities of voltage-dependent ion channels, required to achieve a speed of action potential propagation of several m/s. Beyond the remarkable contribution brought to the discovery of different ion current components and modeling the cardiac cell electrophysiology [2,3], they remained for decades a preferred system for *in vitro* cardiac pharmacology trials. It has been shown that, compared to papillary muscles or ventricular trabeculae, isolated rabbit Purkinje fibers are far more sensitive in detecting changes in action potential (AP) duration (APD90 – duration from the point of maximum upstroke velocity to 90% return from the peak depolarization to the resting potential) and presence of early afterdepolarizations (EAD) for a variety of drugs, including erythromycin, dofetilide, sertindole, and sparfloxacin [4]. Recently, this classical "golden standard" has been challenged by rapid progress in the field of embryonic stem cell (ESC) and especially induced pluripotent stem cell (iPSC) research. Since the first report in 2006 by the group of Shinya Yamanaka of iPSC generation via transfection of four "pluripotency" factors genes using retroviral vectors [5], multiple technological breakthroughs allowed for substantially higher yields of stem cells derived from differentiated cells like skin fibroblasts, peripheral blood lymphocytes, adipocytes or hair follicle keratinocytes, lower risks of tumorigenesis, as well as increasingly sophisticated protocols for redifferentiation of these stem cells into mature cardiomyocytes [6]. The painstaking *in vitro* redifferentiation process requires a stepwise application of conditioning media including three families of extracellular growth factors required for cardiac development in mammals: bone morphogenetic (BMP) factors like BMP4 and activin A, inhibitors of Wnt (wingless) signaling like DKK1 (dickkopf 1), and fibroblast growth factors, like βFGF [7]. However, once obtained, these cardiomyocytes are suitable for a variety of applications, including regenerative medicine approaches, *in vitro* models of diseases, and cardiac safety drug screening.

Multiple pharmacology assays using hiPSC-CM

More than a decade has passed since the first report describing spontaneous APs and contractility in cardiomyocytes within embryoid bodies grown from human embryonic stem cells [8], and recently we have witnessed an emergence of studies using either human embryonic stem cell-derived (hESC-CM) and/or human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) as standardized preparations for *in vitro* experiments. Recent progresses include refinement of cell culture protocols [9], use of antibiotic resistance tags to increase

***Corresponding author:** Amuzescu B, Cytocentrics Bioscience GmbH, Joachim Jungius Str. 9, Rostock 18059, Germany, Tel: +49 381 440 388-0; Fax: +49 381 440 388-47; E-mail: b.amuzescu@cytocentrics.com

Received July 21, 2014; **Accepted** August 15, 2014; **Published** August 22, 2014

Citation: Amuzescu B, Scheel O, Knott T (2014) Novel Automated Patch-Clamp Assays on Stem Cell-Derived Cardiomyocytes: Will they Standardize *In Vitro* Pharmacology and Arrhythmia Research?. J Phys Chem Biophys 4: 153. doi:10.4172/2161-0398.1000153

Copyright: © 2014 Amuzescu B, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Amuzescu B, Scheel O, Knott T (2014) Novel Automated Patch-Clamp Assays on Stem Cell-Derived Cardiomyocytes: Will they Standardize In Vitro Pharmacology and Arrhythmia Research?. J Phys Chem Biophys 4: 153. doi:10.4172/2161-0398.1000153

homogeneity of cell preparations [10], use of retro/lentiviral-free transfection methods in view of clinical applications [6]. Commercial stem cell-derived cardiomyocyte products are available from several suppliers, e.g. hESC-CMs from GE Healthcare (Piscataway, NJ; Cytiva™) or hiPSC-CMs from Cellular Dynamics International (Madison, WI; iCell® Cardiomyocytes), Axiogenesis (Cologne, DE; Cor.4U®), Pluriomics (Leiden, NL; StemCARDcells) or ReproCell (Yokohama, JP; ReproCardio2).

This scientific development led to novel high-throughput pharmacology screening equipments and techniques, based either on optical methods [11], using wide dynamic range Ca^{2+}-sensitive dyes and improved cell loading with extracellular dye fluorescence quenching (e.g. FLIPR® Calcium 6 assay) and transmembrane voltage-sensitive dyes (e.g. FLIPR® Membrane Potential Assay Kit), or multi-electrode array (MEA) methods, allowing extracellular recordings of field potentials (FP), the cellular equivalent of the surface electrocardiogram, impedance measurements [12], and even two-dimensional local activation maps and conduction velocity studies [13]. Both optical and FP MEA-based screening assays present certain advantages, such as high throughput and simplicity of use, the possibility to assess AP propagation and effects of gap junction modulators due to use of confluent monolayers of mature highly differentiated iPSC-CM, and the ability to monitor "subacute" or "chronic" drug exposure effects. However, these systems suffer several shortcomings in exploring the mechanism of action and proarrhythmic effects of certain pharmacological compounds. This is related to the fact that arrhythmogenesis represents an emergent property at cell level, involving complex imbalances between multiple ion current components and intracellular ion handling and modulatory mechanisms [14], and optical/MEA-based iPSC-CM assays report only the sum of all drug-induced effects on the cell. The whole-cell patch clamp adds to this the proper reporting and control of the membrane potential. Recent modeling studies in combination with experimental approaches such as isolated heart preparations have evidenced new arrhythmogenesis mechanisms, such as synchronization of chaotic EADs in the genesis of torsades-des-pointes (TdP) and polymorphic ventricular tachycardia (PVT), a fascinating example of "deterministic chaos" at cellular level [15]. Moreover, the use of detailed cardiomyocyte cell electrophysiology models and advanced mathematical methods like dynamical systems linear stability and bifurcation analysis contributed to a detailed understanding of the mechanisms of cellular pace-making and ectopic focal arrhythmic activity in terms of damped or sustained oscillatory regimes achieved for certain combinations of parameters of a dynamical system, including a steady externally applied current [16-19], which is experimentally possible with whole-cell patch-clamp, but not with extracellular methods like MEA.

Performance of automated patch-clamp platforms for current-clamp AP recordings in hiPSC-CM

Thus, evidence of a pharmacological effect at the level of AP (FP or whole-cell) in spontaneous or induced pacing conditions represents only the first step in a complex process of arrhythmogenic mechanism assessment for that compound. In a simulation study performed with the LuoRudy dynamic model [20], using a large database of 45,000 APs generated with variable combinations of ion current surface densities, in the range of 0.25-2 times the default value, scanned against a test action potential obtained by modifying a single current component, we have shown that the simple AP shape matching criterion does not lead to a unique unambiguous identification of the modified current component [21]. The whole-cell patch-clamp approach offers superior versatility compared to both optical and MEA-based screening assays, because for each tested cell, beyond AP recordings in various pacing conditions, pharmacological effects on different current components can be thoroughly assessed via adequately designed voltage-clamp protocols. There are a few recent reports of hiPSC-CM experiments using planar automated patch-clamp platforms, consisting either in AP recordings in current-clamp mode [22] or voltage-clamp recordings of I_{Na}, I_{Ca} and I_{Kr} [10]. Our own experiments with the CytoPatch™ 2 equipment on iCell® Cardiomyocytes (CDI, Madison, WI) indicate a good stability of AP duration and shape in the classical whole-cell configuration using physiological external and internal solutions, as shown in Figure 1, and the possibility to combine current-clamp and voltage-clamp protocols in complex assays. Previous studies have emphasized the capacity of automated patch-clamp platforms to use very small samples of cell suspension [23], and our special cytocentering technique [24] allows experiments with as few as 150 cells in a 3-µl sample, offering over 80% capture rates and high yields of stable tight seals, with both seal and membrane resistance over 1 GΩ. In addition, using special culture conditions, we obtained routinely high percentages of viable mature cardiomyocytes with predominantly ventricular phenotype (APD50/90>0.7).

iPSC-CMs represent however imperfect electrophysiology models of mature cardiomyocytes of different types, due to variations in the levels of expression of multiple ion channel subunits and other

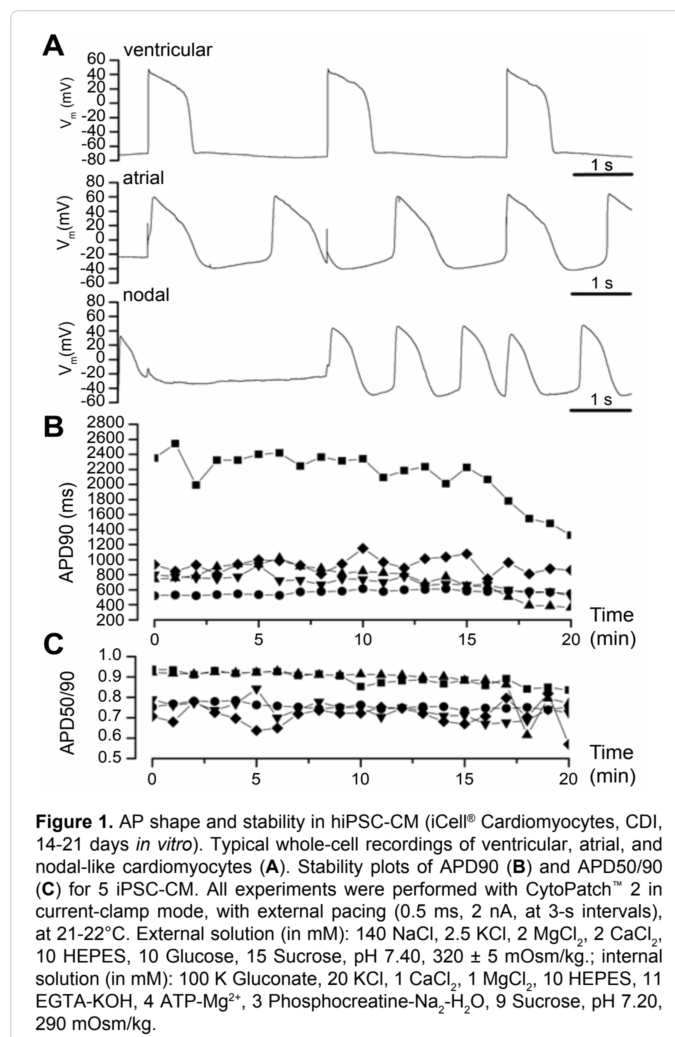

Figure 1. AP shape and stability in hiPSC-CM (iCell® Cardiomyocytes, CDI, 14-21 days *in vitro*). Typical whole-cell recordings of ventricular, atrial, and nodal-like cardiomyocytes (**A**). Stability plots of APD90 (**B**) and APD50/90 (**C**) for 5 iPSC-CM. All experiments were performed with CytoPatch™ 2 in current-clamp mode, with external pacing (0.5 ms, 2 nA, at 3-s intervals), at 21-22°C. External solution (in mM): 140 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, 15 Sucrose, pH 7.40, 320 ± 5 mOsm/kg.; internal solution (in mM): 100 K Gluconate, 20 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11 EGTA-KOH, 4 ATP-Mg^{2+}, 3 Phosphocreatine-Na_2-H_2O, 9 Sucrose, pH 7.20, 290 mOsm/kg.

morphological or functional features, such as a low density of T tubules, which leads to a completely different dynamics of Ca^{2+} between subcellular compartments during an AP [25]. Some of these unwanted features can be corrected using advanced stimulus protocols, e.g. a steady negative externally applied current can override the effects of excessive inward ion currents, bringing the resting potential from a depolarized state back to a value of -80 to -90 mV, specific for mature ventricular cardiomyocytes, and suppressing spontaneous APs, thus allowing pharmacological screening in ventricular-like settings using defined external pacing. Furthermore, a specific current component can be pharmacologically suppressed, while controlling the number of APs executed by the cell, to unravel the repolarization reserve [26], which is also very important in cardiac channelopathies [27].

Multiple ion channel effects and the CiPA paradigm

A paradigm that gains more and more support in the field of drug safety high-throughput screening is that of Multiple Ion Channel Effects (MICE), relying on assessment of pharmacological effects on three ion channels playing the major role in shaping the ventricular AP (Nav1.5, Cav1.2, and hERG1), and then evaluating the risks for different types of arrhythmias (e.g. the "torsadogenic" risk) [28]. Using a receiver operating characteristic (ROC) analysis for different risk prediction models, these authors have convincingly proved that the MICE approach offers a better sensitivity/specificity compromise compared to hERG screening alone, the classical paradigm in cardiac drug safety assessment. The new Comprehensive *in vitro* ProArrhythmia Assay (CiPA) guidelines, issued by a consortium of regulatory agencies and international research institutions, drive home the paramount importance of patch-clamp methods for cardiac safety pharmacology screening, proposing a combination of automated high-throughput or manual voltage-clamp assays and computer simulations with cellular cardiac electrophysiology models, in view of a complex proarrhythmic liability assessment of a drug candidate [29].

The importance of patch-clamp electrophysiology and pharmacology trials is even more obvious when it comes to *in vitro* studies using patient-derived iPSC-CMs as a diagnostic tool in cardiac channelopathies and other cardiac diseases with genetic inheritance [30-33]. Since the clinical picture is often unspecific, an accurate molecular diagnosis currently relies on extensive gene sequencing assays. The use of patch-clamp experiments on patient-derived iPSC-CMs could change the diagnostic and therapeutic flowchart in the future, by pinpointing the faulty ion current component based on significant changes in cell surface density and/or kinetics, thus focusing and limiting the gene screening approach. In addition, patient-derived iPSC-CMs can be used in extensive patch-clamp pharmacology trials to explore in detail the arrhythmogenesis mechanisms, to generate an extended list of drugs incurring arrhythmia risks in an individual case, a list of well-tolerated drugs, and, in certain instances, even a patient-tailored pharmacological treatment for arrhythmia prevention. A summary of our preliminary experiments with patient-specific hiPSC-CM preparations can be found in an application note available at http://www.cytocentrics.com/en-us/newsmedia/downloadinformation.aspx.

Conclusion

In the context of extended electrophysiology and pharmacology trials on human (including patient-specific) iPSC-CMs, automated patch-clamp equipments, such as the CytoPatch™ 2, commercially available at Cytocentrics [24,34], in combination with advanced experimental protocols and computerized modeling studies, may prove invaluable tools in improving the prognosis and life quality of patients with such rare but severe diseases, as well as in routine quality assurance of batch production and daily use of cardiomyocytes in CiPA safety assays.

Acknowledgements

The authors gratefully acknowledge the entire Cytocentrics team for support, and Dr. Blake Anson (Cellular Dynamics International) for instructive discussions.

References

1. Weidmann S (1951) Effect of current flow on the membrane potential of cardiac muscle. J Physiol 115: 227-236.
2. McAllister RE, Noble D, Tsien RW (1975) Reconstruction of the electrical activity of cardiac Purkinje fibres. J Physiol 251: 1-59.
3. Noble D (1962) A modification of the Hodgkin--Huxley equations applicable to Purkinje fibre action and pace-maker potentials. J Physiol 160: 317-352.
4. Lu HR, Vlaminckx E, Teisman A, Gallacher DJ (2005) Choice of cardiac tissue plays an important role in the evaluation of drug-induced prolongation of the QT interval in vitro in rabbit. J Pharmacol Toxicol Methods 52: 90-105.
5. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676.
6. Thorrez L, Sampaolesi M (2011) The future of induced pluripotent stem cells for cardiac therapy and drug development. Curr Pharm Des 17: 3258-3270.
7. Josowitz R, Carvajal-Vergara X, Lemischka IR, Gelb BD (2011) Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders. Curr Opin Cardiol 26: 223-229.
8. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ (2003) Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. Circ Res 93: 32-39.
9. Burridge PW, Matsa E, Shukla P, Lin ZC, Churko JM, et al. (2014) Chemically defined generation of human cardiomyocytes. Nat Methods 11: 855-860.
10. Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, et al. (2011) High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. Am J Physiol Heart Circ Physiol 301: H2006-2017.
11. Burridge PW, Thompson S, Millrod MA, Weinberg S, Yuan X, et al. (2011) A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PLoS One 6: e18293.
12. Himmel HM (2013) Drug-induced functional cardiotoxicity screening in stem cell-derived human and mouse cardiomyocytes: effects of reference compounds. J Pharmacol Toxicol Methods 68: 97-111.
13. Mehta A, Chung Y, Sequiera GL, Wong P, Liew R, et al. (2013) Pharmacoelectrophysiology of viral-free induced pluripotent stem cell-derived human cardiomyocytes. Toxicol Sci 131: 458-469.
14. Noble D (2002) Modelling the heart: insights, failures and progress. Bioessays 24: 1155-1163.
15. Sato D, Xie LH, Sovari AA, Tran DX, Morita N, et al. (2009) Synchronization of chaotic early afterdepolarizations in the genesis of cardiac arrhythmias. Proc Natl Acad Sci U S A 106: 2983-2988.
16. Amuzescu B, Georgescu A, Nistor G, Popescu M, Svab I, et al. (2012) Stability and sustained oscillations in a ventricular cardiomyocyte model. Interdiscip Sci 4: 1-18.
17. Kurata Y, Hisatome I, Imanishi S, Shibamoto T (2003) Roles of L-type Ca2+ and delayed-rectifier K+ currents in sinoatrial node pacemaking: insights from stability and bifurcation analyses of a mathematical model. Am J Physiol Heart Circ Physiol 285: H2804-2819.
18. Kurata Y, Matsuda H, Hisatome I, Shibamoto T (2008) Regional difference in dynamical property of sinoatrial node pacemaking: role of na+ channel current. Biophys J 95: 951-977.
19. Tran DX, Sato D, Yochelis A, Weiss JN, Garfinkel A, et al. (2009) Bifurcation and chaos in a model of cardiac early afterdepolarizations. Phys Rev Lett 102: 258103.

20. Luo CH, Rudy Y (1994) A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes. Circ Res 74: 1071-1096.

21. Corlan AD, Amuzescu BP, Milicin I, Nistor G, Popescu MN, et al. (2009) Feasibility of estimating maximum ion conductance parameters from the shape of the action potential. A simulation study. Biophys J 96: 664a.

22. Stoelzle S, Haythornthwaite A, Kettenhofen R, Kolossov E, Bohlen H, et al. (2011) Automated patch clamp on mESC-derived cardiomyocytes for cardiotoxicity prediction. J Biomol Screen 16: 910-916.

23. Becker N, Stoelzle S, Göpel S, Guinot D, Mumm P, et al. (2013) Minimized cell usage for stem cell-derived and primary cells on an automated patch clamp system. J Pharmacol Toxicol Methods 68: 82-87.

24. Stett A, Burkhardt C, Weber U, van Stiphout P, Knott T (2003) cytocentering: a novel technique enabling automated cell-by-cell patch clamping with the CYTOPATCH chip. Receptors Channels 9: 59-66.

25. Poon E, Kong CW, Li RA (2011) Human pluripotent stem cell-based approaches for myocardial repair: from the electrophysiological perspective. Mol Pharm 8: 1495-1504.

26. Rudy Y (2007) Modelling the molecular basis of cardiac repolarization. Europace 9 Suppl 6: vi17-19.

27. Braam SR, Tertoolen L, Casini S, Matsa E, Lu HR, et al. (2013) Repolarization reserve determines drug responses in human pluripotent stem cell derived cardiomyocytes. Stem Cell Res 10: 48-56.

28. Kramer J, Obejero-Paz CA, Myatt G, Kuryshev YA, Bruening-Wright A, et al. (2013) MICE models: superior to the HERG model in predicting Torsade de Pointes. Sci Rep 3: 2100.

29. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N (2014) Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. Am Heart J 167: 292-300.

30. Blazeski A, Zhu R, Hunter DW, Weinberg SH, Zambidis ET, et al. (2012) Cardiomyocytes derived from human induced pluripotent stem cells as models for normal and diseased cardiac electrophysiology and contractility. Prog Biophys Mol Biol 110: 166-177.

31. Hoekstra M, Mummery CL, Wilde AA, Bezzina CR, Verkerk AO (2012) Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. Front Physiol 3: 346.

32. Knollmann BC (2013) Induced pluripotent stem cell-derived cardiomyocytes: boutique science or valuable arrhythmia model? Circ Res 112: 969-976.

33. Zeevi-Levin N, Itskovitz-Eldor J, Binah O (2012) Cardiomyocytes derived from human pluripotent stem cells for drug screening. Pharmacol Ther 134: 180-188.

34. Scheel O, Himmel H, Rascher-Eggstein G, Knott T (2011) Introduction of a modular automated voltage-clamp platform and its correlation with manual human Ether-à-go-go related gene voltage-clamp data. Assay Drug Dev Technol 9: 600-607.

Citation: Amuzescu B, Scheel O, Knott T (2014) Novel Automated Patch-Clamp Assays on Stem Cell-Derived Cardiomyocytes: Will they Standardize *In Vitro* Pharmacology and Arrhythmia Research?. J Phys Chem Biophys 4: 153. doi:10.4172/2161-0398.1000153

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 350 Open Access Journals
- 30,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscripts as E- mail: www.omicsonline.org/submission/