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Evaluation of Cell Line Identity Using RTCA Profiling for Quality Control of Products Containing Viable Human Cells

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Abstract

Introduction. Identity is an important quality attribute of products containing viable human cells, to be tested during the quality control. The verification of identity includes, among other things, determination of the proliferative activity of the cell lines included in such products. The Agilent xCELLigence real-time cell analysis (RTCA) DP (dual purpose) instrument (USA) for continuous, label-free *in vitro* analysis can be used to assess the cell proliferative activity.

Aim. Demonstration of reproducibility of the RTCA profiling technique as a test method for primary verification of the cell line identity.

Materials and methods. An xCELLigence RTCA DP cell analyzer (Agilent Technologies, USA) was used to obtain RTCA profiles of dermal fibroblast (DF-2) and adipose tissue-derived mesenchymal stromal (MSC AT_D122) cell lines. The experiment was carried out in triplicate after thawing three different vials from the same batch for each cell line.

Results and discussion. The RTCA profiles were obtained for DF-2 and MSC AT_D122 cell lines. The statistical processing of the results was carried out using the Friedman test, confidence intervals, and growth curve parameters obtained by the instrument (doubling time, proliferation rate, and maximum cell index). The obtained data demonstrate no differences in the RTCA profiles after parallel sampling of the contents from three vials for each cell line.

Conclusion. The RTCA profiling reproducibility was confirmed in order to assess the cell analyzer's applicability to cell line identity.

Keywords: cell line, identity, quality control, biomedical cell product, high-tech drug

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Marina A. Vodyakova and Ekaterina V. Melnikova elaborated and designed the experiment and wrote the text of the article. Marina A. Vodyakova, Olga A. Rachinskaya, and Irina S. Semenova performed the experiment. Marina A. Vodyakova and Nikita S. Pokrovsky took part in the data processing. Vadim A. Merkulov provided advice on the results obtained. All the authors took part in the discussions of the article and contributed to the final text of the article.

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Оценка подлинности клеточных линий с помощью RTCA-профилирования для экспертизы качества препаратов на основе жизнеспособных клеток человека

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Резюме

Введение. Одним из важных показателей качества препаратов на основе жизнеспособных клеток человека, определяемых в рамках экспертизы качества, является подлинность. Характеристика подлинности включает в том числе определение пролиферативной активности клеточной линии, входящей в состав таких препаратов. Для оценки пролиферативной активности клеток может быть использован прибор xCELLigence RTCA DP (Agilent Technologies, США), который представляет собой клеточный анализатор в режиме реального времени (RTCA), позволяющий проводить непрерывный анализ *in vitro* без использования меток.

Цель. Показать воспроизводимость методики RTCA-профилирования в качестве метода первичной оценки подлинности клеточных линий.

Материалы и методы. Получали RTCA-профили клеточных линий дермальных фибробластов DF-2 и мезенхимальных стромальных клеток жировой ткани МСК ЖТ_D122 с помощью клеточного анализатора xCELLigence RTCA DP (Agilent Technologies, США). Эксперимент проводили после размораживания трех разных флаконов одной партии каждой клеточной линии в трех повторах.

Результаты и обсуждение. Получены RTCA-профили для клеточных линий DF-2 и МСК ЖТ_D122. Статистическая обработка результатов анализа проведена при использовании критерия Фридмана, доверительных интервалов и параметров кривых роста, полученных с помощью прибора (время удвоения, скорость пролиферации и максимальный клеточный индекс). На основании полученных данных было показано отсутствие различий RTCA-профилей для каждой клеточной линии при параллельном взятии материала из трех флаконов.

Заключение. Обоснована и показана воспроизводимость методики RTCA-профилирования для оценки применимости клеточного анализатора при подтверждении подлинности клеточных линий.

Ключевые слова: клеточная линия, подлинность, экспертиза качества, биомедицинский клеточный продукт, высокотехнологический лекарственный препарат

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. М. А. Водякова и Е. В. Мельникова придумали, разработали эксперимент и написали текст статьи. М. А. Водякова, О. А. Рачинская и И. С. Семенова провели исследование. М. А. Водякова и Н. С. Покровский участвовали в обработке данных. В. А. Меркулов консультировал по полученным результатам. Все авторы участвовали в обсуждении результатов и внесли вклад в окончательный текст рукописи.

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INTRODUCTION

Stringent quality control is a prerequisite for obtaining cell lines with consistent quality characteristics across all the batches of products containing viable human cells (biomedical cell products[1] and high-tech drugs[2]) during manufacturing, quality control, as well as pre-clinical and clinical trials.

The complex composition of products containing viable human cells dictates the necessity to use specific approaches to the cell line quality control. Therefore, it is important to implement new techniques for the determination of particular quality attributes (or sets of attributes), which would involve minimal cell line manipulations.

The real-time cell analysis (RTCA) profiling could be one such technique, performed using the xCELLigence real-time cell analyzer. The instrument's operating principle consists in measuring the cell index (CI) which is proportional to the cell adhesion rate to the electrode surface. The advantages of this technique are non-invasive measurements, the ability to obtain an RTCA profile of virtually any cell line and to record any variation that occurs during the measurement (e.g., microbiological or viral contamination) [1–3].

[1] As defined in the Federal Law No. 180-FZ of June 23, 2016 "On Biomedical Cell Products". Available at: https://minzdrav.gov.ru/ministry/61/23/stranitsa-967/federalnyy-zakon-ot-23-iyunya-2016-g-180-fz-o-biomeditsinskih-kletochnyh-produktah. Accessed: 08.06.2022.

[2] As defined in the Decision of the Council of the Eurasian Economic Commission No. 78 of November 3, 2016 (as amended on March 17, 2022) "On the Rules of Marketing Authorization and Assessment of Medicinal Products for Human Use". Available at: https://docs.eaeunion.org/docs/ru-ru/01431480/err_18032022_36. Accessed: 08.06.2022.

Currently, there are few publications on RTCA profiling for quality control [4, 5], and no publications whatsoever on RTCA profiling for cell line authentication. Thus, RTCA could be proposed as a test method for primary verification of cell line identity based on the comparison of the RTCA profiles, to be used in the quality control of products containing viable human cells.

Aim. Demonstration of reproducibility of the RTCA profiling technique as a test method for primary verification of the cell line identity.

MATERIALS AND METHODS

Cell line culture

The DF-2 (human dermal fibroblast) cell line was obtained from the Russian Cell Culture Collection (RCCC) – a collection of cell cultures of vertebrates maintained by the Institute of Cytology of the Russian Academy of Sciences (RAS). The MSC AT_D122 (adipose tissue-derived mesenchymal stromal cells) line was obtained from the Collection of Cell Cultures for Biotechnological and Biomedical Research (general biological and biomedical fields) maintained by the Koltzov Institute of Developmental Biology of the RAS.

The DF-2 cells were cultivated in the DMEM/F-12 (Gibco, USA) medium with 10% fetal bovine serum (FBS) (Hyclone, USA), 1% penicillin-streptomycin (Gibco, USA). The MSC AT_D122 cells were cultivated in the DMEM/F-12 medium (Gibco, USA) with 10% FBS (Hyclone, USA) and 2 mM L-alanine-L-glutamine (Gibco, USA), 1 % penicillin-streptomycin (Gibco, USA). The cells were cultivated in 5.0 % CO2 at 37 °C and 90 % humidity. The experiments were performed with the cell lines at passage 12.

RTCA profiling

The proliferative activity was determined using the xCELLigence RTCA DP cell analyzer (Agilent Technologies, USA) in a CO_2 incubator (NuAir, Inc., USA). The analysis was performed using 16-well E-plates (Agilent Technologies, USA). For background measurements, 100 µl of the medium was added to each well on the plate and incubated for 30 min at room temperature. After that, at the first stage, 100 µl of both DF-2 and MSC AT_D122 was added, with the concentrations ranging from 2500 to 20 000 cells/well. At the second stage, 100 µl of each cell line was added, with the concentration of 2500 cells/well. The medium with 10 % FSB was added as a control. The E-plates were left at room temperature, and then loaded into the analyzer. The CI was being measured for 120 hrs in the following mode: every 15 min for the first 4 hrs and every 30 min after that. Each cell concentration was measured in triplicate. The experiment was carried out in triplicate after thawing three different vials from the same batch for each cell line.

The CI, the doubling time (DT), which is calculated from the logarithmic growth phase (LOG), and the Slope parameter were calculated automatically by the cell analyzer's software.

Statistical analysis

The statistical analysis of the results and the plotting were performed using OriginPro (v. 2021) (OriginLab Corporation, USA). The normal distribution hypothesis was tested using the Lilliefors-corrected Kolmogorov – Smirnov test. The statistical significance of sample differences was evaluated by the non-parametric ANOVA (the Friedman analysis of variance by ranks) as well as the confidence interval analysis. The differences were considered statistically significant when $p \leq 0.05$. All the data were presented as a mean ± standard deviation, $n = 3$.

RESULTS AND DISCUSSION

Since no products containing viable human cells are currently authorized in the Russian Federation, two cell lines were chosen as model lines for the study. These cell lines have been used in medical practice as part of new medical technologies, or are included in products authorized abroad[1, 2, 3] [6, 7].

The RTCA profiles of the two cell lines at passage 12 were analyzed after thawing three different vials from

[1] Apligraf. Available at: http://www.apligraf.com/ Accessed: 01.10.2021.

[2] LAVIV. Highlights of prescribing information. Available at: https://www.fda.gov/media/80838/download. Accessed: 01.10.2021.

[3] EMA/1380/2018. Alofisel (darvadstrocel). European Medicines Agency, 2017. Available at: https://www.ema.europa.eu/en/documents/overview/alofisel-epar-summary-public_en.pdf. Accessed: 01.10.2021.

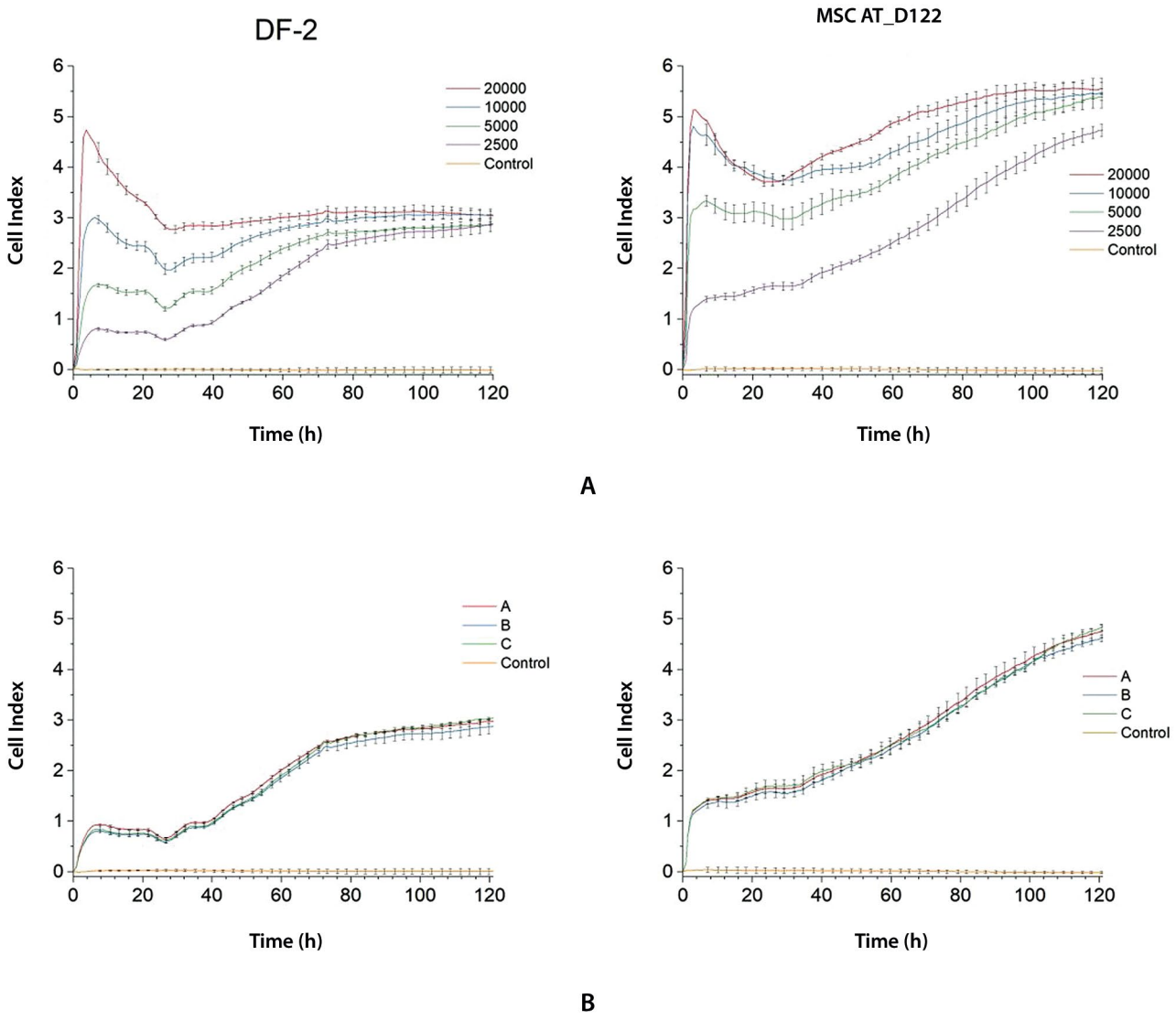

Figure 1. RTCA profiling of DF-2 (left) and MSC AT_D122 (right) cell lines.
A – RTCA profiles of each cell line in a range of different concentrations; B – RTCA profiles of cell lines at 2500 cells/well for vials A, B and C. Data are presented as mean ± SD, *n* = 3

the same batch, in order to evaluate the reproducibility of the technique. The data are presented in Figure 1.

At the first stage (Figure 1, A), the RTCA profiles were obtained for each cell line at different concentrations. It can be noted that both cell lines have a characteristic profile during the lag phase (LAG), which is especially evident at high concentrations, and in the case of DF-2 the CI_{max} was observed at the concentration of 20 000 cells/well. The CI_{max} is a cell index at which the cells reach maximum adhesion to the plate and form a complete monolayer. It can be assumed that such an effect at the adhesion phase is due to the fact that nonadherent cells have a larger size compared to the adherent ones, and the cell size decreased with adhesion.

Further evaluation of the technique's reproducibility was performed with the concentration of 2500 cells/well for both cell lines. At the second stage, the E-plates were inoculated with the samples from three different vials of each cell line at a chosen concentration (Figure 1, B). The RTCA profiles were obtained and quantitatively analyzed using the Friedman test that showed significant differences ($p < 0.05$). However, this result wasn't confirmed by the confidence interval analysis, as the intervals did not differ from one another for the entire curve as well as for the lag

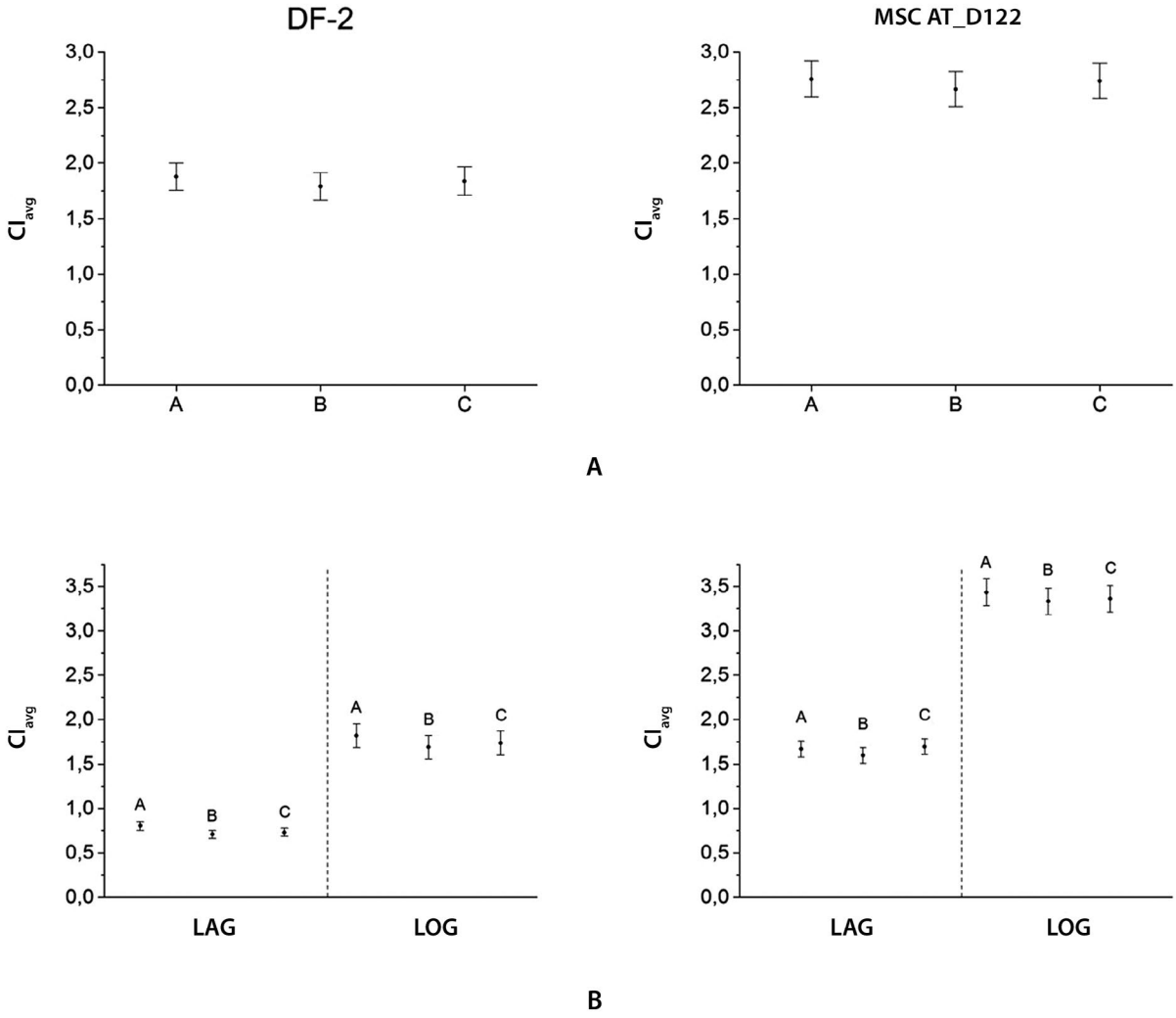

Figure 2. Confidence intervals for cell index calculated:
A – for the entire time interval (120 h); B – for lag and log phases for vials A, B and C

and log phases (Figure 2, B). The differences calculated with the Friedman test could be attributed to the small number of the RTCA profiles. A more precise quantitative estimate would require further comparisons with growth curves obtained for different passages.

Moreover, the RTCA profiles of the three vials from the same cell line were similar in terms of several parameters: DT (39–73 hrs for DF-2 and 54–108 hrs for MSC AT_D122) (Figure 3, A), CI_{max} (Figure 3, B), and Slope (Figure 3, C). The mean DT was 24.5 hrs for the three DF-2 vials, and 54.2 hrs for MSC AT_D122, which is 2.2 times longer. The Slope parameter is defined as the slope tangent to the CI curve and describes the cell proliferation rate. For DF-2, the average Slope was 0.025, and for MSC AT_D122 it was 0.032. Despite the fact that the DT was longer for MSC AT_D122, its proliferation rate was higher. The mean CImax was 3 for DF-2, and 4.7 for MSC AT_D122, which is about 1.5 times higher than for DF-2.

Thus, the confidence interval coincidence, the results of evaluation of the cell line parameters (DT, Slope, and CImax), combined with the visual assessment of the RTCA profiles of the three replicates for the same cell line, make it possible to conclude the absence of significant differences. In addition, despite the fact that dermal fibroblasts are similar in their characteristics to MSC, it should be noted that their RTCA profiles and tested parameters differed significantly. This confirms the possibility of using the technique for primary verification of the cell line identity during quality

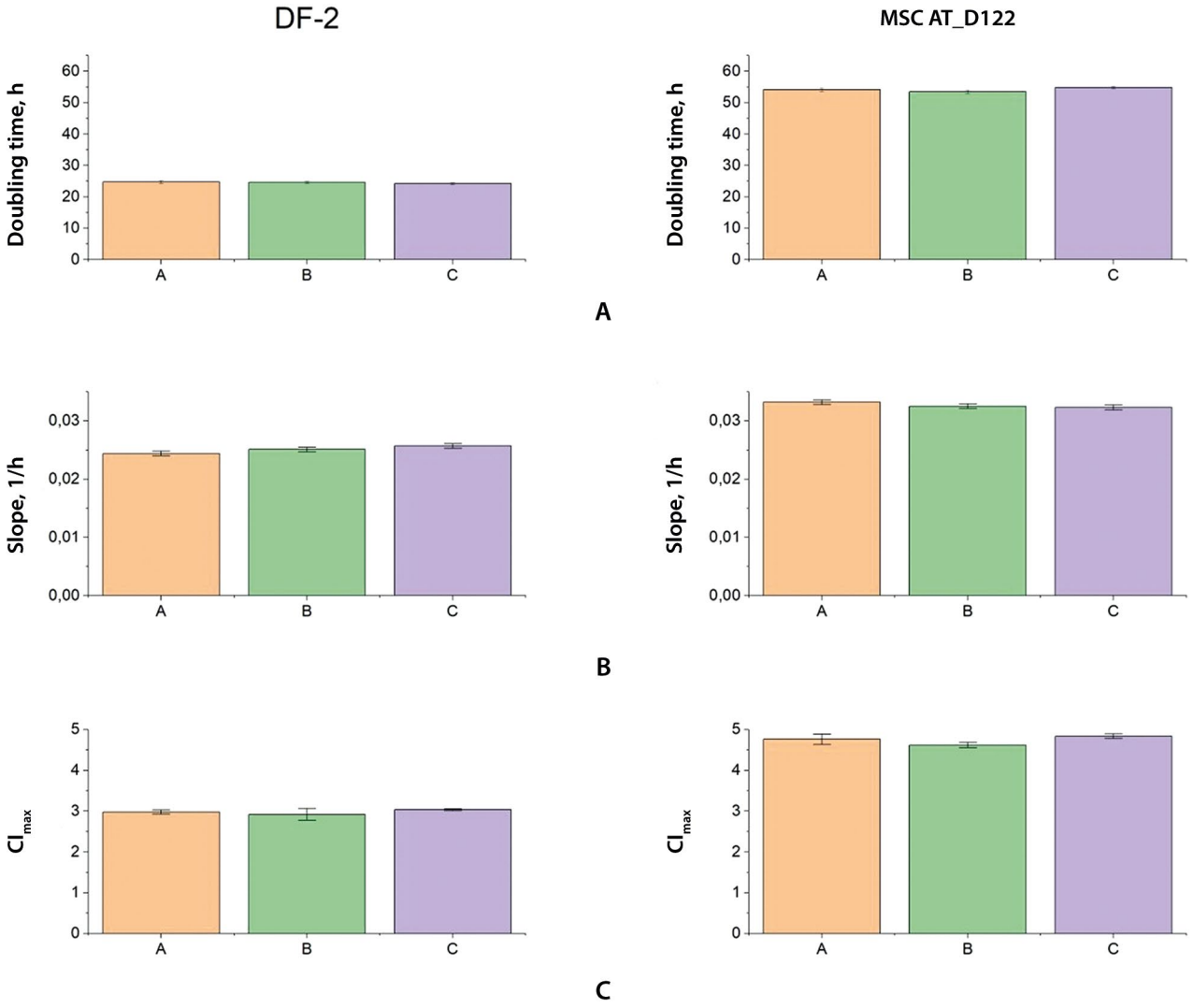

Figure 3. RTCA profiles parameters

A – Doubling time calculated over the period of the logarithmic phase (39–73 h for DF-2 and 54–108 h for MSC AT_D122); B – Slope calculated over 120 h for vials A, B, and C; C – maximum cell index over 120 h for vials A, B and C. Data are presented as mean ± SD, *n* = 3

control of products containing viable human cells. This technique, when combined with phenotypic and genotypic testing, can be used for the evaluation of identity and quality of cell lines included in such products.

CONCLUSION

The RTCA profiling reproducibility was confirmed in order to assess the xCELLigence RTCA DP (Agilent Technologies, USA) cell analyzer's applicability to cell line authentication. The unique kinetic profiles of the DF-2 and MSC AT_D122 cell lines at passage 12, with the concentration of 2500 cells/well, were obtained after thawing three different vials from the same batch and running the analysis for 120 hrs. No differences were observed between the RTCA profiles of each of the tested cell lines.

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