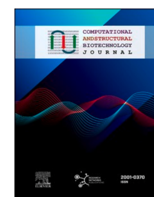
Computational and Structural Biotechnology Journal 23 (2024) 2934–2937

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

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



Short communication

Establishment and identification of bladder cancer cell sheet

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ARTICLE INFO

Keywords:
Cell sheet technology
Bladder cancer
Tumor model
Individualized therapy

ABSTRACT

Cell sheet technology (CST) has primarily been applied in tissue engineering for repair purposes. Our preliminary research indicates that an in vivo prostate cancer model established using CST outperforms traditional cell suspension methods. However, the potential for CST to be used with bladder cancer cells has not yet been explored. In this study, we investigated the ability of two bladder cancer cell lines, T24 and 5637, to form cell sheets. We found that T24 cells successfully formed cell sheets. We then performed staining to evaluate the integrity, specific markers, and proliferation characteristics of the T24 cell sheets. Our findings demonstrate that bladder cancer cell sheets can be established, providing a valuable tool for both in vivo and in vitro bladder cancer studies and for personalized drug selection for patients.

1. Introduction

Cell sheet technology (CST) is a scaffold-free tissue engineering approach that relies on regulating cell culture time and conditions to produce tightly connected, living cell sheets with an autocrine extracellular matrix. This method preserves key surface proteins, such as antigenic epitopes, ion channels, growth factor receptors, and intercellular junction proteins [1,2]. By eliminating the need for exogenous scaffold materials, CST circumvents issues related to immune rejection and ethical concerns. Clinically, epithelial cell sheets have been used to stabilize vitiligo progression [3]; improve survival rates in burn patients through cultured epithelial autografts [4]; and treat corneal limbal epithelial stem cell deficiency with transplanted autologous oral mucosal epithelial cell sheets [5]. CST has extensive applications in tissue repair and reconstruction. However, its potential in oncology remains underexplored.

Our previous work demonstrated the successful culture of DU145 cell sheets, which were used to establish ectopic and orthotopic prostate tumor models, proving superior to traditional cell suspension tumor models [6]. Tumor cell sheets based on CST offer promising applications in preclinical drug development, understanding drug resistance mechanisms, and individualized patient treatment. Bladder cancer is the most prevalent malignant tumor of the urinary system and among the top ten most common cancers globally [7,8]. The development of novel bladder cancer tumor models holds significant promise for advancing new drug discovery and enabling personalized treatment strategies for patients.

In this study, we aimed to establish bladder cancer cell sheets, verify their integrity, assess the stability of cell surface markers, and evaluate their proliferative potential. This work provides a preliminary foundation for developing CST-based bladder cancer models.

2. Materials and methods

Human bladder cell lines T24 and 5637 were obtained from the ATCC and were cultured in PMRI 1640 (Product No. 11875119, Thermo Fisher, United States). All the cell lines were supplemented with 100 U/ml streptomycin/penicillin (Product No. 15140148, Thermo Fisher, United States) and 10 % FBS (Fetal Bovine Serum) (Product No. A5669701, Sigma-Aldrich, Germany) at 37 °C in a humidified atmosphere of 5 % CO_2. Based on our previous experience, cell sheet culture was performed with 5×10^6 cells per 3.5 cm dish, serum concentration was elevated to 20 %, and the rest of the conditions remained constant [6]. The fresh culture medium was replenished daily and the sheets were harvested after 4 days [6]. HE, Masson, immunohistochemical staining and scanning electron microscopy were performed according to our previous experimental protocol [6]. The antibodies used were anti-CK20 (Product No. A19041, ABclonal, China, 1:100), anti-CK7 (Product No. A12004, ABclonal, China, 1:100), anti-Ki67 (Product No. A23722, ABclonal, China, 1:100), and HRP Goat Anti-Rabbit IgG (Product No. AS014, ABclonal, China, 1:100).

3. Results

As depicted in Fig. 1, we seeded 5×10^6 cells into each 3.5 cm dish,

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https://doi.org/10.1016/j.csbj.2024.07.009
Received 14 March 2024; Received in revised form 5 July 2024; Accepted 5 July 2024
Available online 9 July 2024
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increased the FBS concentration to 20 %, and replaced the fresh complete medium daily for four consecutive days. Upon secreting sufficient extracellular matrix, the cells formed a sheet. By placing the cells on a pre-cooled carrier stage and evenly blowing the cells from the periphery to the center, the cell sheet detached completely, as illustrated in Fig. 1. This confirms that T24 cells can successfully form cell sheets suitable for tumor model applications.

We next assessed the cell sheet's integrity and tumor marker signatures to ensure the preservation of its original characteristics. HE and Masson staining revealed that the cell sheet maintained intact cellular structures and included extracellular matrix components such as collagen fibers (Fig. 2). Immunostaining for CK20, CK7, and Ki67 indicated that the bladder cancer cell sheet retained characteristic cellular markers and proliferative potential (Fig. 2). Thus, the cell sheet preserves the hallmark markers and extracellular matrix of bladder cancer cells, validating its use for further research.

4. Discussion and conclusion

The majority of tumor models currently utilized in clinical practice are cell suspension models and the more recently developed organoid models. While cell suspension models are easier to implement, they consist of isolated cells lacking extracellular matrix and intercellular connections, communication, and interactions. Organoid models more closely mimic the actual tumor microenvironment. However, their clinical application is limited by technical complexity and high cost.

In contrast, the cell sheet model offers significant advantages over traditional cell suspension models. It bypasses the need for enzymatic digestion, allowing the direct harvesting of multilayered cell sheets replete with autocrine extracellular matrix. This preservation of ion channels, cytokine receptors, and extracellular components is beneficial for maintaining cell viability and minimizing cell loss and apoptosis post-transplantation. Our prostate cancer cell sheet model has demonstrated superior fidelity to real tumor conditions compared to the cell suspension model, underscoring its potential for enhanced clinical relevance [6]. In addition, J. Yang et al. found that cell sheet-based multilayered liver tumor models can screen anti-cancer drugs and have made significant progress in the field of liver cancer tumor models [9]. Lee J et al. tested the sensitivity of chemotherapeutic agents by in vitro culture of head and neck cancer cell sheets [10]. Meanwhile, Lee J et al. also developed 3D tumor models using cell sheets can be used for in vitro observation of epithelial cancer growth and invasion, as well as anti-cancer drug testing [11]. In conclusion, the tumor model based on cell sheet has important clinical application value.

During the construction of cell sheets from two bladder cancer cell lines, we observed that T24 cells successfully formed cell sheets with a

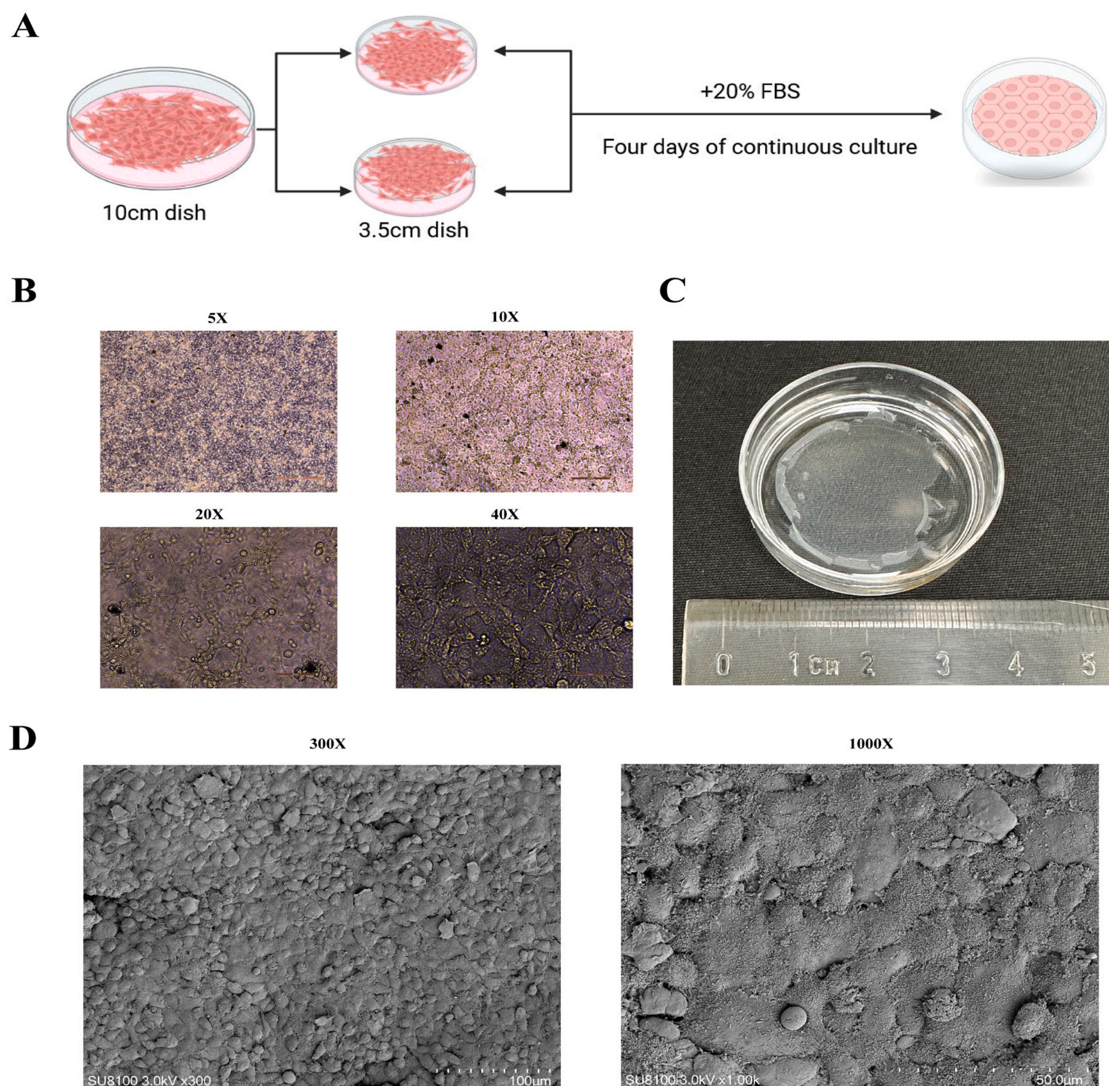

Fig. 1. The process of making a cell sheet. (A) Flowchart for making a thin cell sheet. (B) Microscope view of cell sheet. (C) Intact bladder cancer cell sheets. (D) Scanning electron microscopy images of bladder cancer cells sheet.

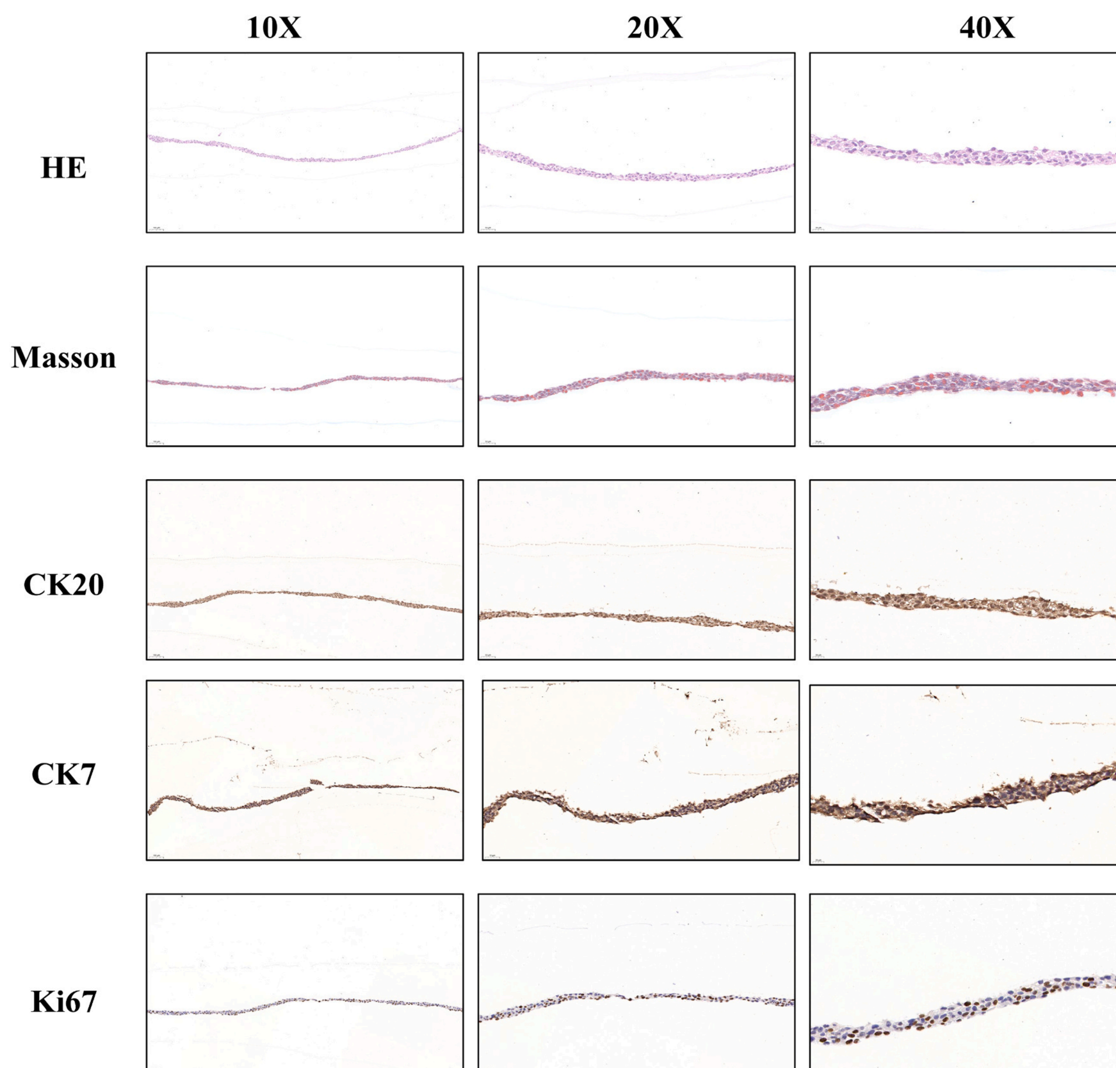

Fig. 2. HE, Masson, CK20, CK-7, and Ki67 staining of bladder cancer cell sheet.

success rate exceeding 80 % using the standard protocol, whereas 5637 cells consistently failed to form cell sheets. We attribute this discrepancy to inherent cellular characteristics. Specifically, we found that 5637 cells were significantly more resistant to enzymatic digestion, requiring over five times longer digestion than T24 cells. This suggests that 5637 cells may secrete higher levels of adhesion proteins. Future work will focus on optimizing the cell sheet formation process to accommodate a broader range of cell types.

While this study successfully established human bladder cancer cell sheets, we have not yet validated the superiority of this model. Further experiments are necessary to substantiate its advantages over existing models.

In conclusion, this study is the first to demonstrate the successful production of bladder cancer cell sheets using CST. This novel tumor model holds potential for applications in drug sensitivity testing and personalized therapy for bladder cancer patients.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by the Shanghai "Science and Technology Innovation Action Plan" star project (23YF1433800), Shanghai Jiao Tong University Medical Engineering Cross Fund (YG2022QN071), and National Natural Science Foundation of China (82172920).

CRediT authorship contribution statement

Tuanjie Guo: Writing – review & editing, Writing – original draft, Methodology. **Zhihao Yuan:** Resources, Methodology, Investigation. **Jinyuan Chen:** Investigation, Formal analysis. **Dongliang Zhang:** Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Xiang Wang:** Supervision, Resources, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

None.

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