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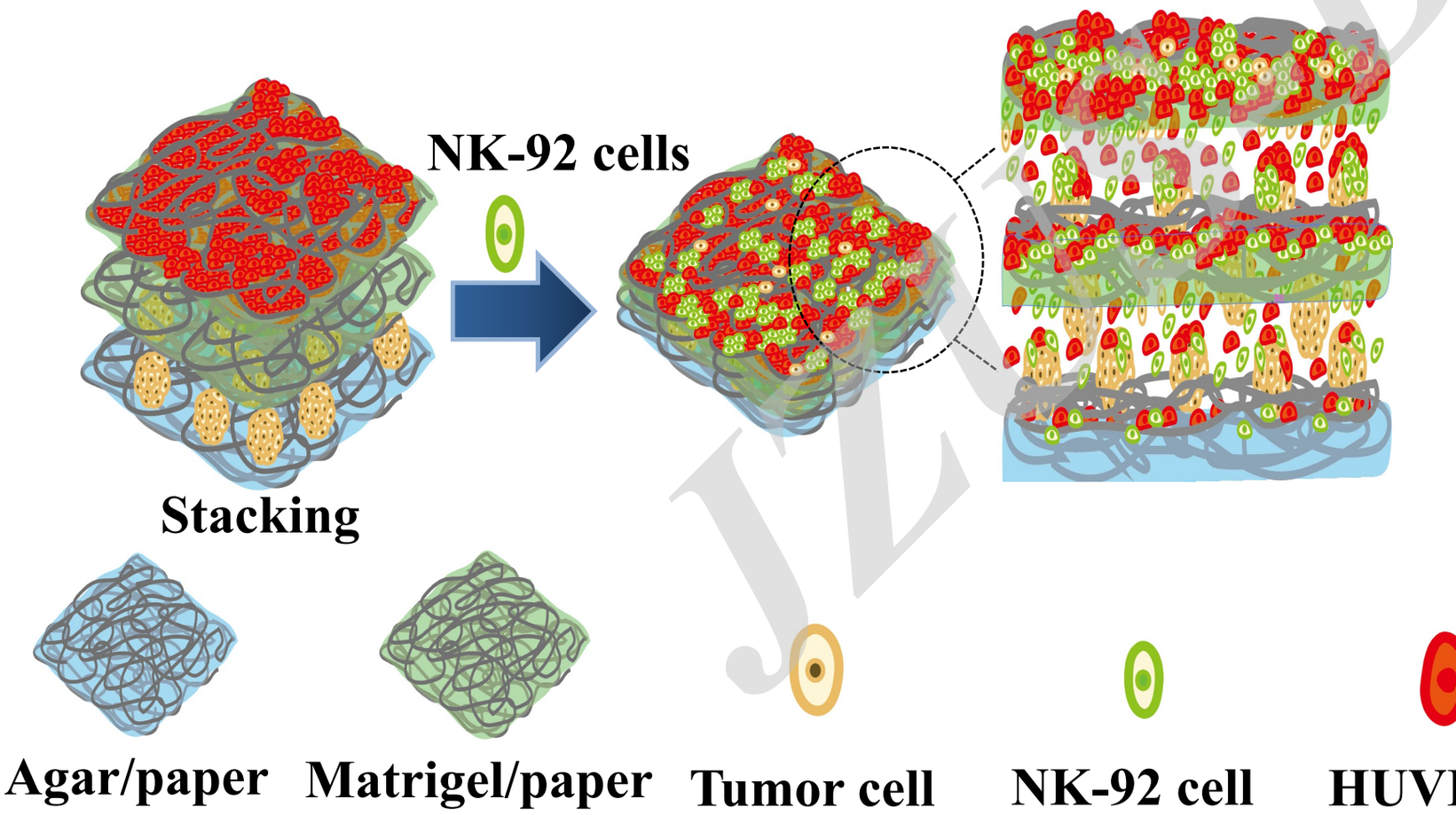
Co-culture of natural killer cells and tumor spheroids on a heterogeneous multilayer paper stack

Key words: Multilayer paper stack; Co-culture; Tumor spheroid; Human umbilical vein endothelial cell (HUVEC); Natural killer cell (NK cell); Migration

Innovation point

Utilizing a heterogeneous multilayer paper stack to create a 3D microenvironment that models the movement of natural killer (NK) cells as they traverse an endothelial layer to target tumor spheroids

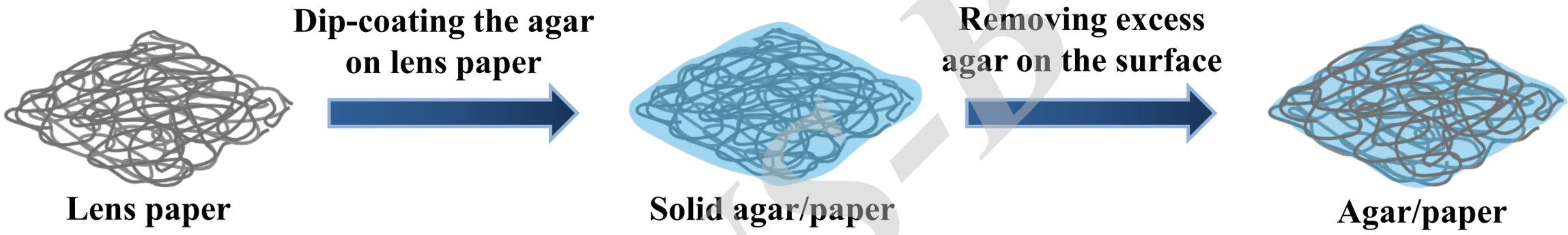


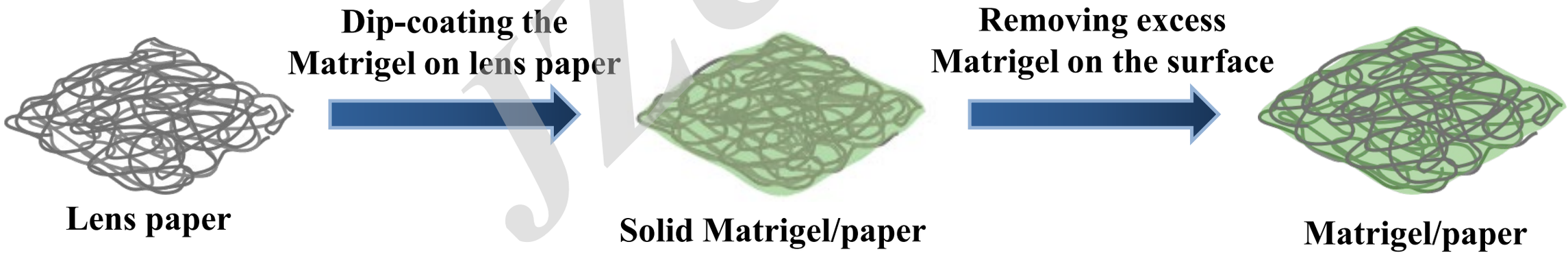
- High transparency
- Easy cell recovery
- Easy to perform multi-cell culture
- Multiple experiments can be performed on the platform

Schematic illustration of cell co-culture in the multilayered papers

Experimental methods

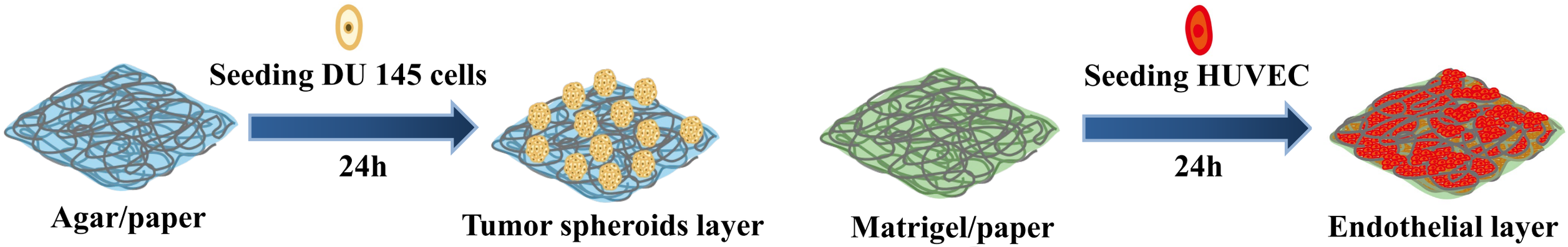
1. Preparing the gel-paper hybrid scaffolds





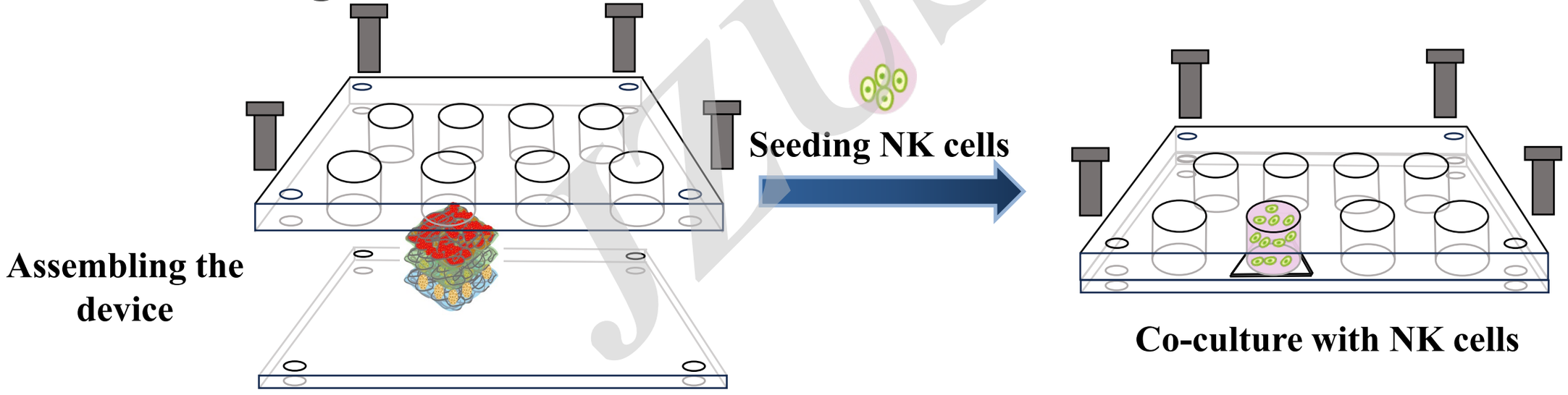
- ✓ The agar/paper combination allows for direct observation under a light microscope and simplifies the collection of tumor spheroids, serving as the bottom layer to support tumor spheroids growth.
- ✓ The Matrigel/paper facilitates cell invasion and acts as the middle layer for cellular invasion.

2. Generating tumor spheroids layer and endothelial layer



- ✓ Tumor cells gather into spheroid on the agar/paper scaffold, with a diameter of approximately 100 μm.
- ✓ HUVECs cover the surface of the Matrigel/paper, forming an endothelium barrier after 24 h of culturing.

3. Co-culturing with NKs in the device



- ✓ The tumor spheroids grown on the bottom agar/paper layer, the blank Matrigel/paper served as the middle invasion layer, and the HUVECs seeded on Matrigel/paper was top endothelium layer.
- ✓ Tumor spheroids migrated from the bottom layer to the middle invasion layer.
- ✓ Some NK cells crossed the top endothelial layer to interact with the tumor spheroids in the middle layer, but only a few NK cells reached the bottom agar/paper layer.

Significance and application

Significance:

- **Provides a novel platform for the 3D co-culture of tumor cells, endothelial cells, and immune cells.**
- **Establishes a model for natural killer (NK) cells traveling across the endothelial layer to form tumor-infiltrating NK cells.**

Three-dimensional (3D) cultures enable cells to retain their morphological and phenotypic characteristics observed in vivo, making them invaluable for studying complex cellular behaviors and interactions. Multilayered paper-based cell culture, as an in vitro 3D cell culture method, has been used to study drug bioavailability, and dose-limiting toxicity in malignant tumors. In this study, we developed a heterogenous multilayer paper stacking co-culture system to model natural killer (NK) cells crossing the endothelial layer to attack tumor spheroids. This system consists of three layers: a bottom tumor-spheroid layer, a middle invasion layer, and a top endothelium layer. NK-92 cells were placed in the supernatant above the three layers. After two days of co-culture, the attack of tumor spheroids by NK cells and the formation of tumor-infiltrating NK cells (TINKs) were observed. The proposed device established a model of NKs traveling across the endothelium layer to form TINKs, which holds promise for further investigations of the interaction dynamics of immune cells and cancer cells.