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The effect of porosity and stiffness of glutaraldehyde cross-linked egg white scaffold simulating aged extracellular matrix on distribution and aggregation of ovarian cancer cells

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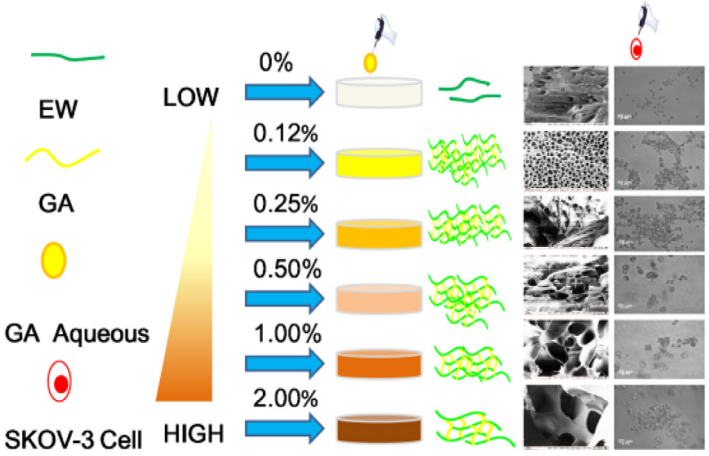
HIGHLIGHTS

- Ovarian SKOV-3 cancer cells could form into multicellular aggregates (MCS) in GA cross-linked egg white (EW) with higher elastic modulus.
- The higher elastic modulus G′ of EW is more beneficial to the proliferation and migration of SKOV-3 cells.
- The degradable GA cross-linked EW is potential 3D cell culture matrix economically.

GRAPHICAL ABSTRACT



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ABSTRACT

The extracellular matrix (ECM) regulates cellular behaviours in a way that it is still far from completely understood, especially in cancer research field. Here we showed a simple system based on different glutaraldehyde (GA) cross-linked egg white (EW) that supported growth of cells on three dimensional (3D) scaffolds. The reaction between GA and EW was highly active. The pore distribution in GA cross-linked EW was more uniform. The mechanical properties of GA cross-linked EW can be tuned through changing the amount of added GA. Overall, the EW based scaffolds were promising tools that may be used as tunable 3D materials in vitro platforms for cancer tissue engineering and anticancer drugs resistance testing.

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1. Introduction

The tumour microenvironment is very complex, because the body cell is wrapped by an extracellular matrix (ECM), and the interaction between cells and the matrix is in the dynamic. A lot of signal molecules and cytokines in which are highly involved in cancer cells growth [1–3]. Compared to the cancer cells in the body, the traditional two-dimensional (2D) cultured cells *in vitro* showed a significantly lower malignant degree of behaviours. The related properties such as cell differentiation, polarity, intercellular communication and contact with the ECM are also altered, so it does not represent the cell in its natural state in the body [4–6]. Modern techniques were developed to study the action of tumor microenvironment in tumor occurrence, development and anticancer drug testing, which indicated that tumor tissue engineering may become an important development direction of tissue engineering [7–9].

The seed cell used for three-dimensional (3D) culture is in the core field of tumour tissue engineering research. Human cancer cells do not experience apoptosis, so this can also result in the infinite proliferation and preferred growth of cancer cells in blood vessels from autocrine signaling [10,11]. Biological materials in tumour tissue engineering play an alternative to the ECM or for the base of the tissues and organs. At present, biomaterials used for 3D culture were numerous, including the ECM, polymer material and inorganic material [12]. The ideal supportive engineering material has the following features: (1) not poisonous—it has good biological histocompatibility and does not trigger immunological rejection; (2) porosity—it has good surface activity, maintains the growth of the cell morphology and phenotype, provides appropriate spatial distribution to the cells; (3) biodegradable—it has plasticity and has a certain mechanical strength; and (4) is in favour of cell adhesion and proliferation—it induces directional differentiation [13–15].

The ECM of different tissues in the human body of different patients in different age groups which features such as the mechanical property of its physical state has obvious difference [16]. Histologically, solid tumours also demonstrate different degrees of brittle lesions at different stages [17]. The architecture, composition and stiffness of ECM *in vivo* were further subject to changes during disease progression and aging. Cancer is closely associated with aging of the body. In addition, tumor formation *in vivo* is accompanied by a progressive stiffening of the tissue and ECM, as evidenced by the finding that mammary tumor tissue and tumor-adjacent stroma are between 5 and 20 times stiffer than normal mammary gland, respectively. Local microenvironments simulated a medium material impact on the behaviors of the cancer cells in order to guide the tumour prevention and treatment [18].

Natural materials with superior biocompatibility are used for tissue engineering [19]. EW is a kind of medium containing water, with protein as colloidal substance of its dispersed phase, which accounts for about 60% of the total weight of eggs; 85% to 89% is water, and, of the rest of the solids, about 80% is protein [20]. As the cell culture medium, EW contains the complete nutritional and signal components of development that are needed, its mechanical properties and morphology can be widely modified [21,22]. EW especially easily could be obtained economically. This is its advantage that other biological materials do not have [23].

Cross-linking refers to the internal interaction between the molecules through a covalent bond, and also including the role of signal molecules in increasing the tension and the stability of the biological materials [24]. It included physical cross-linking and chemical cross-linking. However, chemical cross-linking has a high cross-linking efficiency, degradation resistance is better [25].

Glutaraldehyde (GA) was most commonly used in gelatin. Meanwhile, it is cheap relatively. In addition, it can cross-link with gelatin completely in low concentrations and improve its mechanical properties [26]. According to reports, GA cross-linked gelatin materials

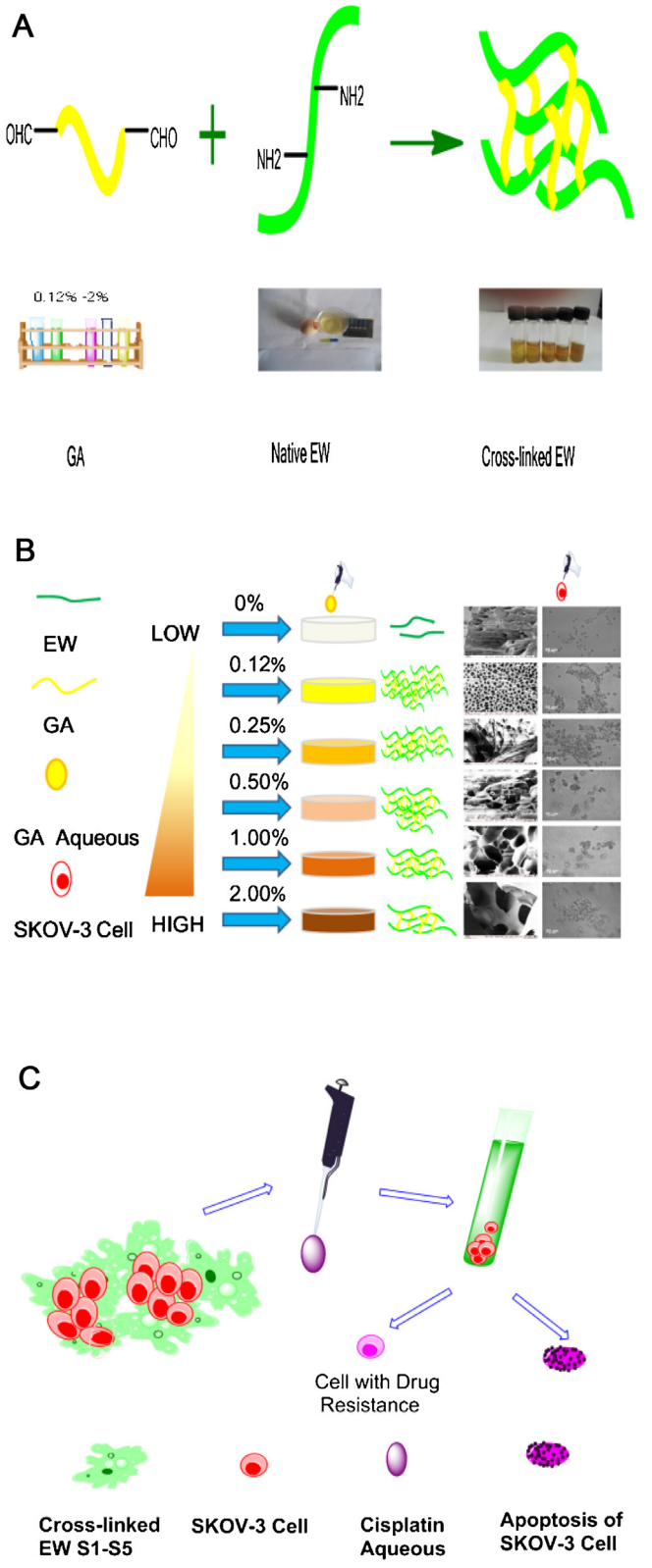

Fig. 1. (A) Cross-linking reaction of polymer containing amine group with GA in EW (PH 7.6–8.5 approximately); (B) schematic demonstration of the effect of the porosity and stiffness of GA cross-linked EW simulating aged ECM on the aggregation of ovarian cancer cells; (C) the effect of stiffness of scaffolds on anticancer drug resistance of cells.

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