Free-Standing Cell Sheet Assembled with Ultrathin ECM as an Innovative Approach for Biomimetic Tissues

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Statement of Purpose: Current artificial tissue-substitutes have limited clinical applications due to unmatched complex combination of cells and extracellular matrix (ECM) as seen in native tissues. From a developmental perspective, the construction of effective biomimetic tissues is from the bottom (one-dimensional nanoparticles or two-dimensional membranes) up (three-dimensional scaffolds or more complex composite). In a hierarchical architecture, each sub-structure can be assembled in a flexible way with specific regulators and cells, which overcomes the deficiency of one-for-all scaffold. Here, a cell-compatible cell-lined layered nano-membrane is developed. Bioactive molecules are mounted on a nano-membrane and later released to its lined cell sheet. The cell-lined membrane is in a free-standing form to regulate cellular functions. The major advantage of this methodology is to provide a versatile approach to construct biomimetic tissues for clinical applications.

Methods: The glass slides, N-isopropylacrylamide (NIPAM) and 3-acryloxypropyl- trimethoxysilane (APTES) were purchased from Aldrich Chemical Co. All solvents were purchased from VWR Canada.

Glass Substrate was pretreated and its surface was modified by silane, followed by surface-initiated graft polymerization with NIPAM. The surface modified glass wafers was then characterized. After that, human C2C12 cells were cultured onto the PNIPAM-grafted slides. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) was conducted subsequently. LBL assembly of gelatin-(chitosan-alginate)3 on a monolayer of cells was also prepared and characterized. Finally, the free-standing LbL assembly of cell-gelatin-(chitosan-alginate)3 was prepared and characterized.

Results: We developed a cell-compatible free-standing cell-lined layered nano-membrane. Bioactive molecules were mounted on a nano-membrane and later released to its lined cell sheet. The cell-lined membrane can be a free-standing form to regulate cellular functions, and also can be assembled with different types of cells. The major advantage of this approach is to provide an alternative and flexible approach to construct biomimetic tissues for the application in translational medicine.

The contact angles of the surfaces after different steps were measured to evaluate the layer-by-layer deposition of the various polyelectrolytes. The morphology of a free-standing cell-nanomembrane is shown in Figure 1B,C.

To evaluate the compatibility of the nanomembranes on cells, the live/dead assay of the gelatin–(chitosan/ alginate)3 nanofilms on the cell surface was conducted. The result shows 99% cell viability, revealing the important role of gelatin as the first layer next to the cells. The immunostaining images also present a monolayer of healthy cells.

To investigate the effects of regulator-loaded membranes on the osteogenic differentiation of stem cells, a free-standing membrane composed of BMP2-loaded gelatin-(chitosan/alginate)3 membranes lined with mouse BMSCs was fabricated and characterized. It was demonstrated that the long-term ability of enhancing the osteogenic differentiation of mouse BMSCs and the freestanding soft property make it very suitable for the potential application of tissue regeneration.

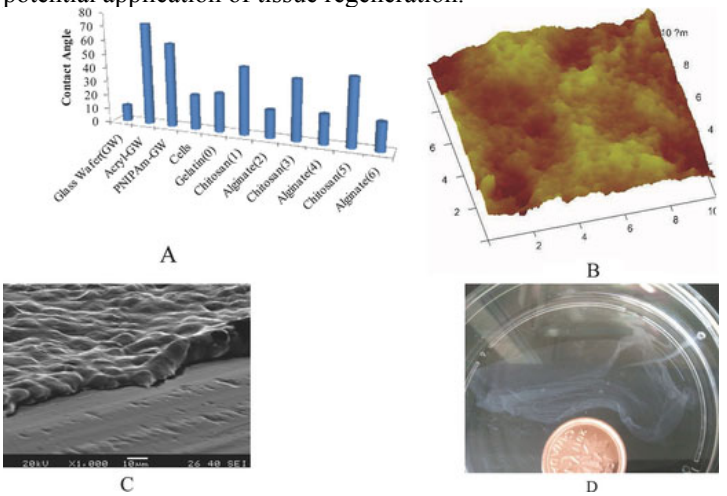

Figure 1. A) Contact angles of various surfaces in preparing the free-standing film. B,C) AFM and SEM images of a free-standing nanomembrane-cell sheet. D) Photo of free-standing film floating in a PBS buffer.

Conclusions: A novel strategy to fabricate a free-standing multilayer nanomembrane lined with a cell sheet was developed in the present work. The complex film could be peeled from the substrate upon temperature changes to form a free-standing planar cell-nano membrane. The architecture was well organized, even after the complex was peeled off the substrate. The scaffold mechanically supported the cell sheets and maintained the cell proliferation at the minimal cost of adding extra polymer components on the cells. This feature may point out the way out of the dilemma existing in the traditional scaffold, minimizing the ratio of the functional cells to the synthetic materials to obtain the greater overall functions. The gelatin–(chitosan/alginate)3 assemblies exhibited good cell compatibility to meet the fundamental requirement for the consecutive application of the tissue construction. Most significantly, the ability of nanomembrane to control the release of loading drugs and growth factors provided us a potent approach to control the cell differentiation in the scaffold. To the best of our knowledge, this is the first effort to fabricate free-standing, cell-lined, and multilayered membrane via layer-by-layer deposition as an ECM to regulate cell differentiation. The planar free-standing nanomembranes can be potentially used as “bricks” and “concrete” at same time to fabricate various layered complex assembly with target cells and regulators to prepare complex soft tissues.

References: Chen J. et. al. Adv. Funct. Mater. 2014;
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