Chinese Yam-Derived Adhesive for Tissue Engineering

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Statement of Purpose: Chinese yam is wdiely used in traditional Chinese remedies and has been reported to aid in neuroprotection, amelioration of amnesia, antioxidant protection, and fungicidal activity in clinical studies. Its extracted polysaccharide helps T cell growth and proliferation [1]. This herbal medicine is rich in sticky mucin and adhesive proteins, which make it easy to coat a variety of materials. While fat and other componnets are reported, the Chinese yam is mainly composed of protein and polysaccharide, which mimics the charactersitic of extracellular matrix [2]. The above characters, combined with its nutritional and biocompatible properties, make the extract an attractive candidate for tissue engineering. In this study, the extract from the Chinese yam was coated onto different substrate surfaces.The nanostructure was examined using atomic force microscopy (AFM). We observed that the extract could form a network of 10 nm nanofibers. These fibers formed a porous scaffold with 40-50 nm pores. 3D analysis showed that the porosity was greater than 70%, which provides sufficient space for cell growth and attachment. Next, we evaluated the potential for this material to be used in tissue engineering. Cell attachment was tested by seeding HeLa cells onto surfaces coated by the extract. PolyHema was used as a negative control. This in vitro test indicated that the network provides an excellent framework for cell attachment and growth. The identification of this novel network will impact further research into using plant derived scaffolds for tissue engineering.

Methods: Chinese yam was bought from Sunrise, a local supermarket at Knoxville, TN. The yam was cut and the sticky pulp from cutting surfaces was collected gently using 10 ul pipette tips. Repeated cutting and collection steps until an around 500 ul of solution was collected. The collected solution was then centrifuged at 15,000 g for 1 hour, and the supernatant was collected by pipette tip to a new 1.7 ml eppenderf tube. The pellet was washed with 20 nm – filtered water. The structure of supernatant and the resuspended pellet were then coated to the coverslip and examined by Agilent 5500 AFM system for morphology. For cell attachment, the supernatant adhesive and the resuspended pellet was coated to the 24-well plate pre-coated with polyHEMA. HeLa cells were then seeded at $5x10^4$ per well, and were incubated at 37°C for different period of time. Cell attachment and cell growth were examined under light microscopy.

Results: To understand the structure necessary for tissue engineering, we first examined the nanoscale morphology of the supernatant adhesive from Chinese yam. Figure 1 showed the nanofiber-formed scaffolds from different scale. These nanofibers uniformly spread across the area that was examined, which were embedded with sparse of particles in different size. These nanofibers formed a shape of scaffold with necessary character for cell attachment. These nanofibers have a diameter of around 10 nm which formed network with pore size of around 40 nm. In the resuspended pellet, we also examined the nanoscale morphology and no similar networks were detected.

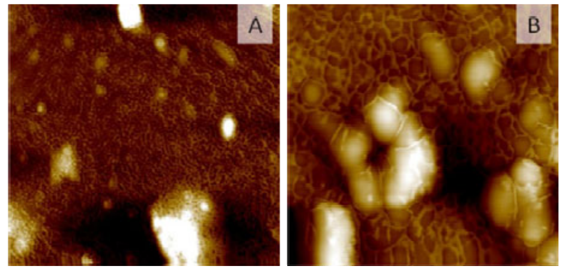

Figure 1: Morphology of extracted liquid adhesive from Chinese yam. A) AFM image of extracted adhesive at 2x2 um. 8) AFM image of extracted adhesive at 0.76x0.76 um.

In the next, we evaluated the cell attachment of the scaffolds formed from the adhesive. HeLa cells were shown adhering to the network coated wells, but they didn't attach to the polyHEMA coated wells (Figure 2). Although some cells appear to "ball-up" into a spherical morphology, but most cells began to extend or already spread on the surface of the scaffold. The scaffold thus appeared not only optimal for cell attachment, but also for cell proliferation of HeLa cells. Continued examination confirmed the consistent extension of the cells during 72 hour period.

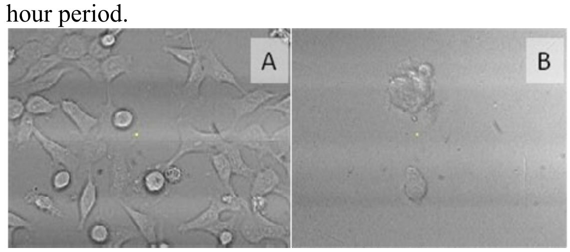

Figure 2: HeLa cell attachment to the liquid adhesive coated surface. A) The surface was coated with polyHEMA, then with liquid adhesive. B) The surface coated with polyHEMA only.

Conclusions: Chinese yam extracted liquid adhesive can be used to make networks with uniform nanofibers and nanopores. The cell attachment study indicated that this network provided an excellent platform for cell attachment, cell growth and proliferation. Cytotoxicity was not observed in coated adhesive. This study suggested a new source of cost-effective material for tissue engineering. The ongoing project involves the isolation and modification of the adhesive to make hydrogel-based adhesive, which leads to the wide application of this plant-based adhesive in tissue engineering and other biomedical fields.

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References:

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2. Yang M. J Ethnopharmacol. 2009;121(1):130-134