Behavior of Dental Pulp Stem Cells on Polyvinyl Alcohol/Halloysite Nanotube Electrospun Nanofibrous Scaffolds

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Statement of Purpose: In modern dentistry, the concept of guided bone regeneration (GBR) is applied at dental implant sites where a barrier membrane/scaffold is used to cover the bone defect to encourage new bone ingrowth while preventing the migration of epithelial cells. Although present polymeric GBR products show positive results in clinics, their weak mechanical properties and poor bone regeneration capacity are still major challenges for material scientists and clinicians. Therefore, a nanocomposite electrospun fiber scaffold seems to be a suitable choice for developing advanced GBR membranes. Polyvinyl alcohol (PVA) is a polyhydroxy polymer that has been studied extensively because of its good film forming and physical properties, high hydrophilicity, biocompatibility. Halloysite nanotubes (HNTs), a kind of natural tubular nanoclay, have been recognized as the additive of polymers for better mechanical properties or thermal properties. We found that HNTs may effectively increase the strength and cell viability of PVA film (Zhou WY, J Biomed Mater Res. 2010; 93A: 1574–1587). Vergaro *et al*. reported the nontoxicity of HNTs (Vergaro VE, Biomacromolecules. 2010; 11: 820-826).

In this study, PVA nanofibers and PVA/HNTs composite nanofibers have been produced by electrospinning and further crosslinked by glutaraldehyde (GA)**.** The structure and properties of these nanocomposite scaffolds were evaluated and discussed. The behavior of human dental pulp stem cells (DPSCs) on PVA and PVA/HNTs scaffolds were tested *in vitro* by means of cell viability, SEM and laser confocal morphologies.

Methods: PVA (2488) was purchased from ChuanWei Group, China. The HNTs were mined from Yichang, Hubei, China. PVA/HNTs composite fibers were produced by electrospinning. The electrospun composite fibers were further crosslinked by GA. The effects of voltage, HNTs contents and GA contents on the electrospinnability were investigated. The diameters of collected fibers were measured by SEM images. Electrospun PVA/HNTs nanocomposite scaffolds were characterized by DSC, TGA, XRD, FTIR and TEM. Dental pulp was extracted from orthodontic patients with ethical approval and the dental pulp cells were cultured by routine method. Stem cells were isolated by an immune magnetic bead selection mouse IgG kit. Prior to cell culture assays, all scaffolds (1x1cm^2) were sterilized by exposure to UV light. DPSCs were routinely removed from tissue culture polystyrene (TCPS) dish and plated on different substrates. Cell viability was evaluated by the CCK-8 assay. SEM analyses were performed to study the morphology of DPSCs at day 4. A confocal laser scanning microscope was applied to observe cell cytoskeletal arrangement and nuclei morphology after stained with fluorescent phalloidin and DAPI dilactate.

Results: For neat PVA nanofibers, the voltage was varied from 12 to 20 kV to find the relationship between fiber morphology and applied voltage. With the increase in voltage, the mean diameter of the fibers increases from 333 to 433 nm. Six different HNTs contents (relative to PVA weight) such as 6, 10, 20, 25, 30 and 40 wt% were examined. After adding HNTs, the fiber diameter decreases sharply to 120 nm. This may be due to the change of suspensions' viscosity by the incorporation of HNTs. The TGA and TEM (Fig. 1) results confirmed that HNTs was successfully loaded in the fiber during electrospinning and the thermal stability was increased with the loading of HNTs. After crosslinking, the morphology of the fibers has not been changed obviously except some conglutination appeared. Importantly, the porous morphology of scaffold, which is crucial for tissue regeneration, was retained.

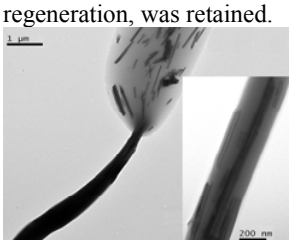

Fig. 1 TEM images of PVA/HNT nanocomposite nanofiber.

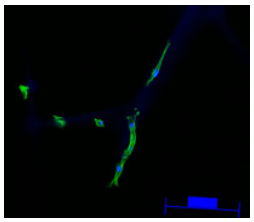

Fig. 2 Confocal image of DPSCs on PVA/HNTs (6%) scaffolds at day 4, bar=200 μm.

All the CCK-8 value of PVA and PVA/HNTs scaffolds increased with culture times which showed a desirable biocompatibility. Neat PVA scaffolds had lower CCK-8 value compared with TCPS at all culture times. High polar O-H group on neat PVA and the use of GA crosslinker may account for the lower CCK-8 value. For nanocomposite scaffolds, the HNTs seem to favor for the attachment and proliferation of DPSCs when the content of HNTs below 10%. At day 4, there is no significant difference of CCK-8 values between neat PVA and PVA/HNTs scaffolds except of 25%. DPSCs formed a 3D spherical structure (spheroids) on neat PVA scaffolds by SEM and confocal observation. Confocal image (Fig. 2) showed that on nanocomposite surface, some DPSCs displayed the organized cytoskeletons with defined actin microfilaments and distinguished focal contacts. Spheroids were still existed on PVA/HNTs composite scaffolds. Stem cells from dental pulp showed both 2D and 3D growth phenomena on PVA/HNTs nanocomposite fibrous surface.

Conclusions: PVA/HNTs nanocomposite fibers have been produced by electrospinning for GBR application. The HNTs/PVA composite nanofibers have diameters in the range of 100-400 nm. The nanocomposite structure was confirmed by TGA and TEM. The crosslinking of the fiber did not change the morphology significantly and the porous characteristics of the scaffold were retained. DPSCs can grow both in 2D and 3D (spheroids) on PVA/HNTs nanocomposite scaffolds up to 7 days.