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Characterization of genipin crosslinked Wharton's jelly properties for use in bone regenerative medicine

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Tissue engineering offers the ability to repair injuries that the body fails to heal. The use of perinatal tissues is of increasing interest in tissue engineering because they have a privileged immune status as well as anti-inflammatory, antifibrotic and antimicrobial properties. In addition, these tissues are a valuable source of surgical waste. Among them, the Wharton's Jelly (WJ), extracted from the umbilical cord, is mainly composed of collagen fibers, proteoglycans, hyaluronic acid and contains many growth factors. Its low nucleic acid content (less than 50 ng / mg of dry tissue) allows its use as an allogeneic graft. Nevertheless**,** its low mechanical properties limit its use in bone regenerative strategies. Herein, our objective is to crosslink WJ in order to improve its strength and thus its potential use as a bone regenerative medicine strategy.

Umbilical cords are collected after delivery and stored at -20 ° C. WJ membranes (1 x 5 x 12 mm) were obtained after removal of blood vessels and amniotic tissue. They were washed, lyophilized, and stored at -20 ° C. These membranes are crosslinked in genipin solution with different mass ratios (i.e. 0.05 - 0.1 - 0.2 mg of genipin / mg of dry tissue) for 24 hours in D-PBS at 37 ° C. Increasing levels of crosslinking degrees (i.e. 40 - 60 - 90%) were demonstrated by ninhydrin assay. FTIR analysis showed a prominent peak at 1732 cm^{-1}, confirming the incorporation of genipin into WJ-membranes. A decreasing swelling rate compared to uncrosslinked membranes was demonstrated (i.e. 25%, 40% and 55% depending on the increasing degree of crosslinking). The mechanical properties were evaluated under hydrated conditions on a tensile bench. The elasticity modulus increases as a function of the crosslinking degree (i.e. uncrosslinked WJ = 0,51 MPa ; crosslinked 40% WJ = 1,32 MPa ; crosslinked 60% WJ = 1,95 MPa : crosslinked 90% WJ = 2,61 MPa) . Resistance to degradation was assessed by a collagenase digestion test (1 mg / mL for 72 hours). The percentage of degradation varies from 40% to 0% depending on the increasing crosslinking degree. Comparatively, uncrosslinked membranes was completely degraded after 72 hours. The cytotoxicity of crosslinked WJ membranes were evaluated according to ISO.10993-5 (i.e. Mitochondrial activity and release of lactate dehydrogenase) against mesenchymal stem cells (MSCs), osteoblasts and fibroblasts. No cytotoxic effect has been demonstrated. The proliferation of MSCs were followed for 21 days of culture on crosslinked WJ-membranes demonstrating a reduction in proliferation in comparison with uncrosslinked membranes. Finaly, the implantation of the 90% crosslinked WJ membranes at rat calvaries bone defects showed a better osteogenic potential after 8 weeks compared to the implantation of the WJ membranes.

In conclusion, these results show that a membrane can be developed from Wharton's jelly. Its mechanical and degradation properties can be improved by crosslinking with genipin without inducing a cytotoxicity effect. However, the percentage of crosslinking has an influence on the adhesion of cells to the membranes. At last, the osteogenic potential of the 90% crosslinked membranes appears promising, but the results need to be confirmed by histology and densitometry study.