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Mechanical characterization of cell-seeded nano-cellulose scaffolds developed for auricular cartilage engineering

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Like any cartilaginous tissue, the external part of the ear, the auricle, cannot regenerate once damaged. Tissue engineering (TE) is a potential alternative to surgical reconstruction which would reduce donor site-morbidity. Adequate mechanical properties which allow daily activities (e.g. sleeping, wearing earphones) are key for successful ear reconstruction. Various biodegradable ear-shaped scaffolds have been investigated in the past, yet, due to inadequate degradation rates, mechanical properties deteriorated with long-term *in vivo* implantation. Nano-cellulose (NC) is a biocompatible but non-biodegradable biomaterial, making it a promising scaffold material for ear reconstruction. This study aims to characterize the mechanical properties of various seeded and unseeded NC scaffolds and compare them to native human auricular cartilage in order to decide on the best candidate for auricular TE.

**Materials and Methods**

Four different NC scaffolds were produced by bacterial synthesis: non-porous 15%-NC (85% water, 13 samples), non-porous 10%-NC (13 samples), non-porous 5%-NC (13 samples), microporous NC (>99% water, 8 samples). Auricular chondrocytes were cultivated on the microporous NC scaffolds for 6 weeks (8 samples). Cell-seeding is not possible on non-porous NC, because chondrocytes cannot penetrate in the cellulose scaffold. Additionally, fresh human auricular cartilage samples were harvested during reconstructive surgery (39 samples) or post-mortem (42 samples) with approval of the local ethics commission. Both NC and human samples were cut to 5x5 mm², and tested in stress-relaxation indentation (5%-strain steps with 30 minutes relaxation) on a Zwick-Z005 machine (10 N load cell, Ø0.9 mm indenter). Material properties, including equilibrium modulus ($E_{eq}$), were computed using MATLAB.

**Results**

Native auricular cartilage has a higher $E_{eq}$ (2698.0 ± 1498.4 kPa) than 15%-NC (2446.1 ± 401.8 kPa), 10%-NC (1188.2 ± 211.6 kPa), 5%-NC (233.2 ± 103.6 kPa) and cell-seeded microporous NC scaffolds (24.7 ± 10.6 kPa). The stiffness of unseeded microporous NC scaffolds was below the sensitivity of the testing system.

**Discussion and Conclusions**

NC mechanical properties are highly tunable, covering a stiffness range from < 15 kPa up to 2400 kPa, where 15%-NC scaffolds have the greatest mechanical potential as a candidate for auricular TE. Cell-seeding and subsequent extracellular matrix production improves the mechanical properties of the microporous scaffolds in comparison to unseeded microporous scaffolds, yet these are still mechanically inferior to both non-porous and native auricular cartilage. In future, the combination of high concentration NC with a microporous structure and TE cell culture will be used to increase scaffold strength and facilitate tissue production, making them a relevant choice for auricular TE efforts.

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