Chondrogenesis of mesenchymal stem cells using electrospinning

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S**tatement of propose:** Electrospinning as a fabricating method, is not a new method, but has however become a handy manufacturing process, which allows the manufacturing of continuous fibers with nanoscale diameter for tissue engineering scaffolds. Human adipose-derived stem cells (hASCs) are multipotent stem cells that can be differentiated into fat, bone, and cartilage. They can be easily/safely obtained from adipose tissue, regardless of the age and sex of the donor. hASCs can be maintained and expanded in culture for long periods of time without losing their differentiation capabilities, making them a primary choice for cell therapy purposes. Electrospraying/spraying live cells have been introduced in the recent years as a cell seeding method but changes in the phenotype and genotype of the cells has not been studied. In this study the cells will be directly incorporated into a polymer solution prior to Electrospraying. Cells that are exposed to this process differentiate into chondrocytes without any exogenous factors. Multiple previous studies have demonstrated the effects of various chemical factors, such as soluble growth factors, chemokines, and morphogens, on chondrogenesis. In particular, transforming growth factors (TGF-β) and bone morphogenetic proteins (BMPs) have been shown to play essential roles in the induction of chondrogenesis. Although growth factors have great therapeutic potential for cartilage regeneration, growth factor-based therapies have several clinical complications, including high dose requirements, low half-life, protein instability, higher costs, and adverse effects. The present data suggest that electric signaling has high potential as adipose derived mesenchymal stem cells-based therapy for cartilage regeneration.

Materials and Methods: P2-P4 of adipose tissue-derived stem cells (hASCs) from Lonza (Walkersville, MD, USA) were used for cell cultures. Cells were plated in T75 culture-treated flasks with approximately 1 million cells per flask, and culture media was changed every 3-4 days for the duration of the culture. The gelatin/pullulan solution with the final concentration of 1.25mg/ml was used for electrospinning. Cell electrospinning content was aseptically transferred to a sterile 10 ml syringe, and a sterile 18-gauge syringe needle tip was secured. The collector plate, which is a petri dish (Fisherbrand, polystyrene)), was positioned 7.5 cm from the end of the needle tip. The syringe pump settings were adjusted to produce readings for a plastic 10 ml syringe pump. The pump rate was set to 200 μL/min. electrospraying was performed at 10 and 15 kV. Control experiment was performed without applying any voltages. RNA sequencing libraries were prepared using TruSeq Stranded mRNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA) according to the manufacturer`s protocol. The libraries were quantified using the Promega QuantiFluor dsDNA System on a Quantus Fluorometer (Promega, Madison, WI). The size and purity of the libraries were analyzed using the High Sensitivity D1000 Screen Tape on an Agilent 2200 TapeStation instrument. The libraries were normalized, pooled and subjected to cluster and pair read sequencing was performed for 150 cycles on a HiSeqX10 instrument (Illumina, Inc. San Diego, CA, USA), according to the manufacturer's instructions.

Results and Discussion: Using standard electrospinning conditions at 10kV, [1]we examined the effect of voltage on hADSC differentiation to assess cell differentiation. Production of glycosaminoglycan (GAG) on the grown culture was analyzed based on Alcian Blue absorbance at 650 nm. Both the absorbance value of Alcian Blue stained cells in 10kv and 15kv increased gradually from day 7 to 14. To reveal the differentiation associated with phenotype change, a DEG analysis was performed to identify gene expression changes between electrospun and control samples. Chondrogenic markers such as matrilin 4 (MATN4), aggrecan (ACAN), collagen type VI alpha 3 chains (COL6A3), collagen type IX alpha 1 chain (COL9A1), and SRY-box 6 and 9 (SOX6 and SOX9) were upregulated as early as at day 7.

Conclusions: Result from this study suggest that this technique can be an alternative method for differentiating adipose-derived mesenchymal stem cells into chondrocytes to ultimately produce usable cartilage.

References:

1 Nosoudi, N., et al., *Electrospinning live cells using Gelatin and Pullulan.* Bioengineering, 2020. **7**(1): p. 21.