



IN VITRO CHONDROGENIC CAPACITY CLASSIFICATION OF JUVENILE CHONDROCYTE DONORS FROM CULTURED PELLET SIZE

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Regenerating cartilage is a challenging problem affecting millions of Americans. Cartilage cells (chondrocytes) do not regenerate well, either inside or outside the body. Chondrocytes from human donors can be grown in cultures, either as pellets or sheets to implant into damaged cartilage. Average pellet area, both in CSTEC's preliminary data and external publication^[1], shows promise as a measurable, donor-linked characteristic for chondrogenic potential. We demonstrated that the human cytokine BMP6 facilitates cultured pellet size enlargement from human donors (Figs. 1, 2). Additionally, pellet culture source (i.e., cell sheets formed into pellets or conventional pellet culture) appears to affect final cultured pellet size (Fig. 2). Human donor variability appears to play the largest role in determining cultured pellet size, with donor 18 warranting further examination (Fig. 2). From these data, all external factors examined so far apparently affect cultured chondrocyte pellet size.

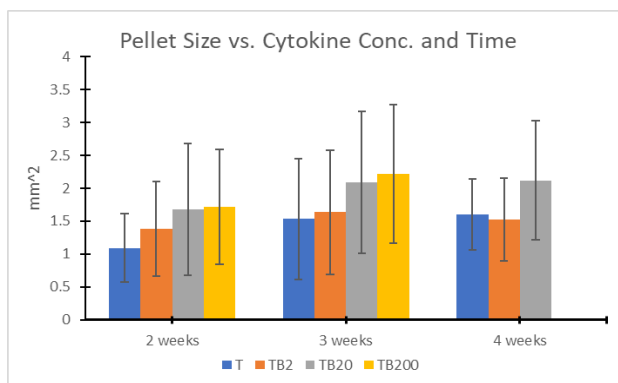


Figure 1: Average human chondrocyte cultured pellet area by differentiation time and media cytokine concentration. TGF- β 3 was at known native concentration in all groups. T: TGF- β 3 alone, TB: TGF- β 3 combined with BMP6. TB("number") indicates BMP6 concentration in ng/mL. Pellets tend to increase in area with BMP6 concentration and plateau in size at 3 weeks; TB20 and TB200 3-week

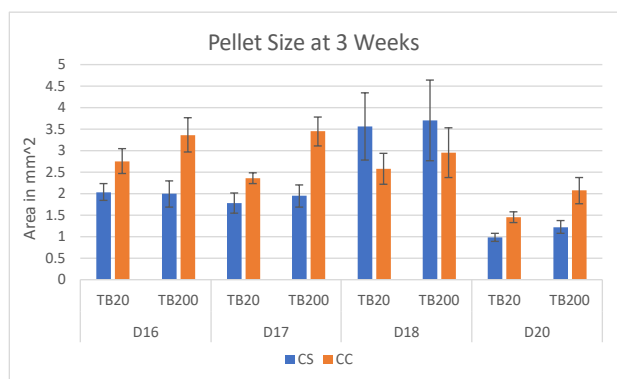


Figure 2: Average cultured human chondrocyte pellet area from 3-week cultured pellets from various human donors. Donor chondrocyte cells tend to form the largest pellets across donors and BMP6 concentrations. However, Donor 18 is an exception to both trends.

1. Sekiya, I., et al., *In vitro* cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc. Natl. Acad. Sci.*, 2002. 99(7): 4397-4402.