LARGE-SCALE PRODUCTION OF ACPC-DERIVED CARTILAGE ORGANOIDS

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Introduction

Current treatments for articular cartilage defects often lead to fibrous repair tissue with insufficient mechanical properties. Furthermore, the supply of autologous chondrocytes is limited.[1] Spinner flask culture of chondrocytes supplemented with notochordal cell-derived matrix (NCM) can produce ECM-rich cartilage organoids.[2] However, the amount of organoids is still limited by the initial number of chondrocytes. A promising alternative is articular cartilage progenitor cells (ACPCs), which can be expanded up to 30 passages without dedifferentiating.[3] Therefore, the aim of this study was to develop a method to fabricate clinically relevant amounts of cartilage organoids using ACPCs, and to assess the quality of the mini tissues.

Methods

Human ACPCs were obtained from residual cartilage tissue from total knee replacement surgeries from the Maxima Medical Center (METC, number N16.148) following established protocols.[3] For pre-aggregated groups, 250.000 cells were seeded. Spinner flask culture followed the protocol by Crispim & Ito.[2] When mentioned, constructs were stimulated with one dose of 100 ng/ml of BMP-9 (day 1-3). After 14 days, GAGs (DMMB assay) and DNA (Qubit,ThermoFisher) were quantified. Alician blue staining was performed. A parallel (unconfined) compression test was performed (MicroTester G2, Cell Scale). Young's modulus was calculated as the slope of stress/strain curve (0-20% strain). Statistical analysis was carried out using Prism (Graphpad). Multiple comparisons were assessed with a one-way ANOVA, followed by Bonferroni correction post-hoc t-test.

Results

BMP-9 stimulation synergized with spinner flask culture, lead to a significant increase in diameter (>200%, not shown) ($p>0.0001$) and GAG/DNA when compared to static culture and to using only NCM in the spinner flasks (Figure 1.a). NCM aggregated samples stimulated with BMP-9 presented more matrix than samples simply self-assembled in the stirrer flasks with BMP-9 alone (Figure 1.b), and were stiffer (Figure 1.c).

Discussion

In this study, BMP-9 stimulation improved cartilage matrix production, as shown by Morgan *et al.*[4] In contrast to Crispim and Ito, using only NCM with ACPCs in the spinner flasks did not stimulate proliferation and it was not fully incorporated into the organoids, resulting in smaller organoids.[3] Unlike chondrocytes, ACPCs may be less responsive to NCM, but also incorporated NCM could have interfered with cell-to-cell interaction necessary for ACPC maturation. However, when NCM was incorporated with cell proliferation, this lead to a more functional stiffer organoid. Therefore, upcoming experiments will focus on production of ACPC cartilage organoids by enabling self-assembly and stimulating ECM production by combining BMP-9 with other additives. This system has the potential to facilitate the fabrication of clinically relevant numbers of high quality organoids for cartilage repair.

References

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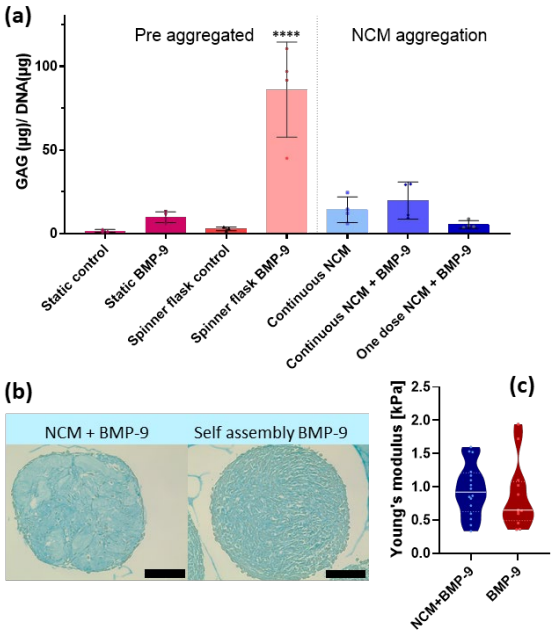

Figure 1: GAG/DNA was significantly higher for pre aggregated constructs stimulated with BMP-9 (a). More ECM in the organoids (b) translated to higher Young's modulus (c).