



Effect of 3D bio-extrusion and polymerization processes during cartilaginous substitutes production using alginate-based bio-ink

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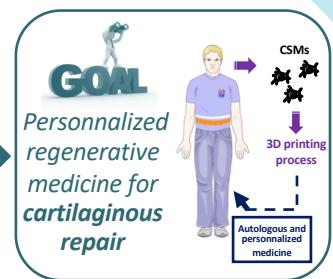
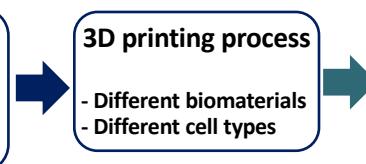
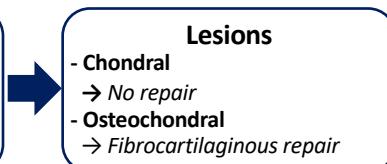
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Introduction



Tissue engineering : Biomaterials → Cells → Culture conditions → Evaluation of cell viability

Objectif

Evaluation of the effect of 3D printing bio-extrusion and polymerization processes on the mitochondrial activity of human bone marrow mesenchymal stem cells (hBM-MSCs) to produce cartilaginous substitutes containing 1 million (1M) or 2 millions (2M) of cells/ml.

Methods

Human Bone Marrow from osteoarthritis patients

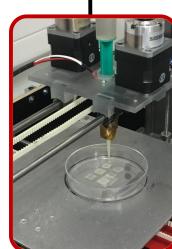
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Mesenchymal Stem Cells (MSCs)

Alginate Bio-ink
1 or 2 million cells / mL

- ITS (minimum medium) or
- TGF- β 1 (chondrogenic medium)

3D printing process

Bio-extrusion



Polymerization



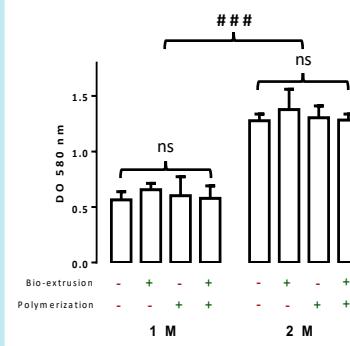
Mitochondrial activity studies (MTT test)

D0, D3, D7, D14, D21 and D28



Results

Mitochondrial activity of the MSCs at Day 0 :



Results : No significant effect of the bio-extrusion and of the polymerization steps has been highlighted, regardless of cell density. Furthermore, the cell mitochondrial activity measured is directly proportional to the quantity of cells seeded in the bio-ink.

Figure 1 : Mitochondrial activity of the MSCs in the different substitutes with a concentration of 1 or 2 M cells/mL. Data are shown as mean ± SD. The effect of the different steps were tested by a two-way ANOVA. Then, the effect of the cells density was globally tested by a one-way ANOVA. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. (n=4)

Mitochondrial activity of the MSCs at D3, D7, D14, D21 and D28 in the conditions ITS or TGF- β 1 :

Results : The mitochondrial activity slightly increases in the first days, similarly for the two cell concentrations. From D7, mitochondrial activity remains stable up to D28 for the two cell concentrations without significant difference between the two culture conditions, ITS or TGF- β 1.

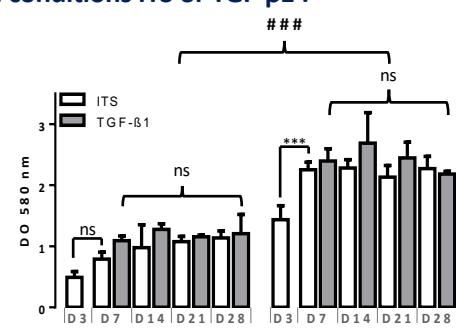


Figure 2 : Mitochondrial activity of the MSCs treated or not with TGF- β 1 over time. Data are shown as mean ± SD for each time, the effect of TGF- β 1 is compared to ITS only by a two-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The effect of the cells density was then globally tested by a one-way ANOVA. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.

Conclusion

The extrusion nor the polymerization step don't have any effects on the cellular activity. The used 3D printing process associating an alginate bio-ink with hMSCs are adapted to produce cartilage substitutes.