3D Printed pectin constructs for the regulation of mesenchymal stem cell differentiation in an endochondral ossification in vitro model

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INTRODUCTION

Endochondral ossification (ECO) is the developmental process underlying long bone formation. It starts from the formation of a cartilage template, which undergoes hypertrophy, finally resulting in vascularization and neo-bone formation [1]. Approaches to mimic ECO mainly rely on the chondrogenic differentiation of progenitor cells on a scaffold, followed by hypertrophy. The final step of vascularization and bone formation is achieved with in vivo implantation. In this study we aimed at investigating how scaffold physicochemical properties influence the first ECO stages. In particular, a pectin-based construct was employed thanks to its biocompatibility, biodegradability and gelling capability [2].

METHODS

Three different pectin-based inks were developed by tuning (3-glycidyloxypropyl)trimethoxysilane (GPTMS) content [3] and by adding nHA (50% w/w) to obtain a range of stiffness. 3D woodpile-shaped constructs were printed and used in combination with fibrin gel (fibrinogen 2.5 mg/ml and 0.5 U/ml thrombin). Scaffolds were characterized by ESEM, and their mechanical properties were confirmed by uniaxial compressive test. Subsequently, a pool of human bone marrow-derived stem cells (hBMSCs), isolated from four different patients, was loaded into the fibrin gel and cultured in chondrogenic medium for two weeks, followed by two further weeks in hypertrophic medium (figure 1) to assess the influence of the scaffold stiffness and composition on cell viability, proliferation and differentiation towards a chondrogenic and hypertrophic phenotype by performing Live/Dead and Alamar Blue assay, matrix deposition quantification and gene expression.

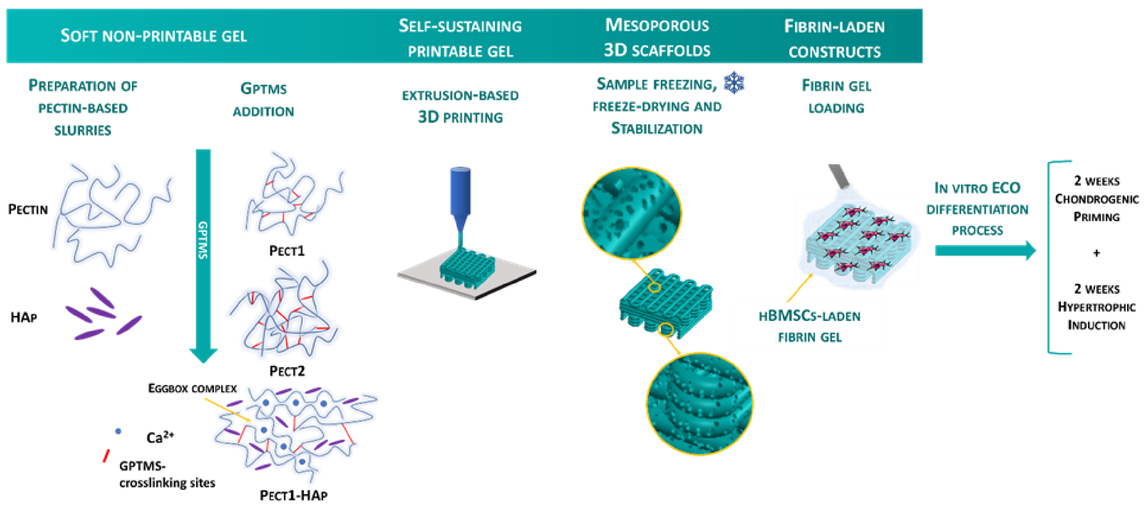

Figure 1- Schematic of the process used to fabricate pectin-based scaffolds loaded with fibrin gel

RESULTS

3D Printing of pectin constructs

All scaffolds showed a good printability that allowed to generate self-supporting woodpile scaffolds (figure 2a) with interconnected macroporosity/mesoporosity (figure 2b), as confirmed by ESEM analysis. Pect1, the pectin scaffold with the lowest GPTMS concentration (0.92 ml/g pectin, Pect1) showed the lowest compressive modulus both alone (48 kPa) and with the addition of the fibrin coating (55 kPa).

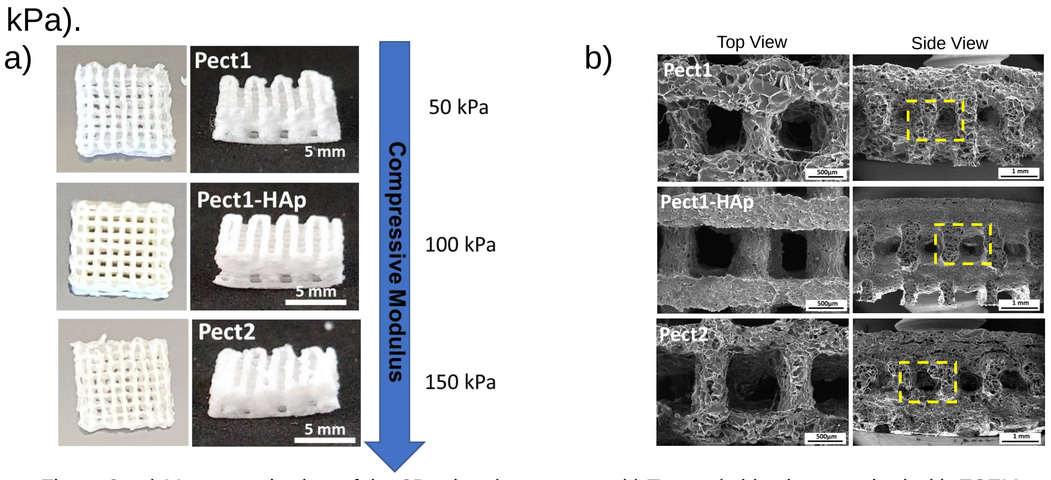

Figure 2 - a) Macroscopic view of the 3D printed constructs , b) Top and side view acquired with ESEM

Cell Viability

hBMSCs embedded in fibrin at 7.5 M/ml were homogeneously distributed on the struts of the scaffold and, additionally, they showed an elongated shape both at 2 weeks in chondrogenic medium and 4 weeks in hypertrophic medium (figure 3b) in presence of fibrin. A poor cell adhesion was observed with pectin only constructs (Pect1 and Pect2). At D1, D4 and D7, the hBMSCs metabolic activity was higher in presence of fibrin embedding, with the highest short-term metabolic activity in Pect1-HAp-F (figure 3a). The addition of a cell-adhesive coating for cell embedding supported both cell proliferation and viability.

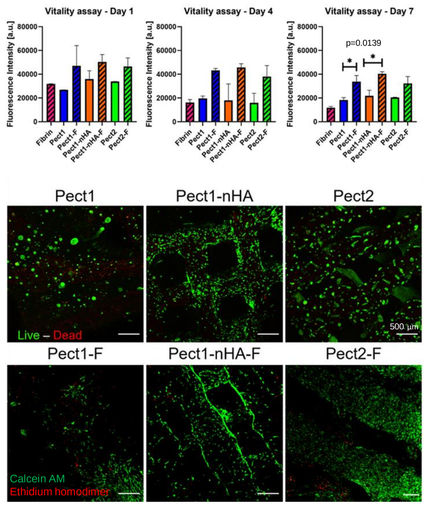

Figure 3 - hBMSCs seeding of Pectin constructs with or without fibrin; a) Short-term Metabolic activity by Alamar Blue, b) Live&Dead assay at 4 weeks

Chondrogenic priming

After 2 weeks of chondrogenic differentiation, hBMSCs seeded on the scaffold with the lowest GPTMS content and with no HA (Pect1-F) produced more GAGs (figure 4a). In particular, GAG/DNA increased from day 1 to day 14, showing the highest value for Pect1F (498.34 µg). Additionally, expressed higher levels of the chondrogenic genes collagen type II (COL2A1), aggrecan (ACAN) and of transcription factor SOX9 (figure 4b), overall suggesting a better chondrogenic differentiation.

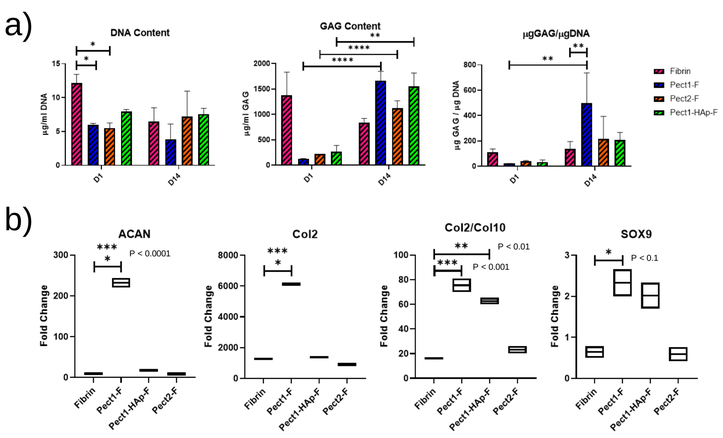

Figure 4 - hBMSCs Chondrogenic differentiation on fibrin-laden constructs scaffolds at week 2; a) Analysis of GAG content for cell-embedded fibrin-laden scaffolds at day 1 and day 14, after chondrogenic differentiation; DNA content evaluation through Cyquant assay, b) Gene expression assessed by RT-qPCR for relevant chondrogenic genes

Hypertrophic induction

Pect1-F showed also the highest expression of the hypertrophic genes as Collagen type 10 (ColX), Matrix Metalloproteinase 13 (MMP13) and Runt-related Transcription Factor 2 (RUNX2) after additional 2 weeks of hypertrophic differentiation (figure 5b). Additionally, the expression of Collagen type X was higher for hBMSCs on Pect1-F scaffolds compared to Pect1-HAp-F and Pect2-F (figure 5a).

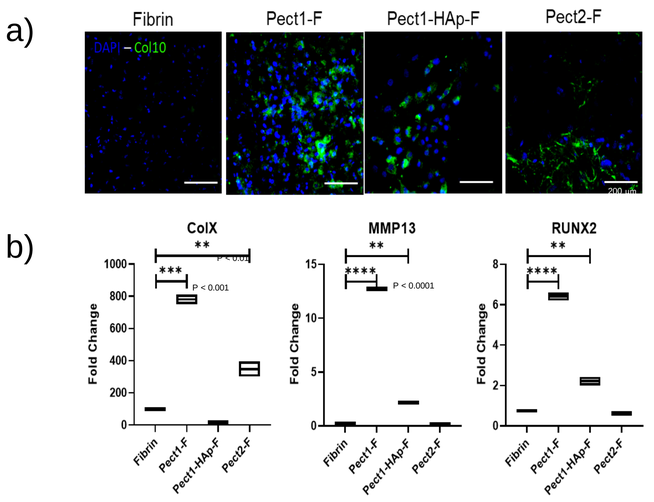

Figure 5 - Hypertrophic differentiation of hBMSCs on fibrin-laden constructs at week 4; a) Immunofluorescence staining for ColX, b) Gene expression assessed by RT-qPCR for relevant hypertrophic genes

CONCLUSIONS

Among the analyzed constructs, Pect1-F mostly supported the formation of a chondro-template at week 2 chondrogenic differentiation followed by an enhanced hypertrophic differentiation. thus Pect1-F can be considered the most suitable candidate for a complete recapitulation of ECO after in vivo implantation. Our findings suggest that a scaffold that will be further implanted should primarily promote the early phases of the ECO process and should not necessarily match the properties of the final bone targeted tissue.

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