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3D-Printing of Platelet Rich Plasma-based Ink for Cartilage Tissue Regeneration

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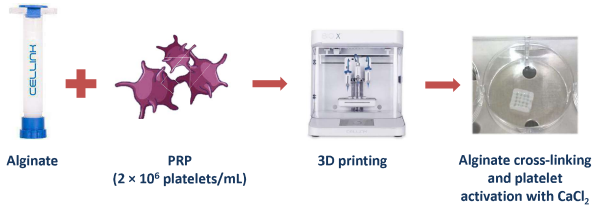
Therapies to regenerate human articular cartilage defects are still challenging due to poor tissue reparative potential and no self-renewing nature of chondrocytes. Among the innovative trends used for cartilage repair, in the last decade, Platelet Rich Plasma (PRP) has emerged as a promising therapeutic agent to promote tissue regeneration, since the growth factors contained in platelet granules trigger proliferative stimulus on human chondrocytes while maintain their somatic differentiation potential [1]. Recently, tissue engineering and regenerative medicine fields are pushing on 3D bioprinting as novel method for the 3D fabrication of living tissue-like structures based on the capability to create cell-laden scaffolds with predesigned architecture and distribution of biological factors [2]. Here we produce a new type of bioactive-ink for 3D printing with the aim of encapsulating human articular chondrocytes in enhanced scaffolds able to promote cellular functions and finally chondrogenesis by supplying a sustained quantity of growth factors.

1. Preparation of PRP- alginate mixed bioink

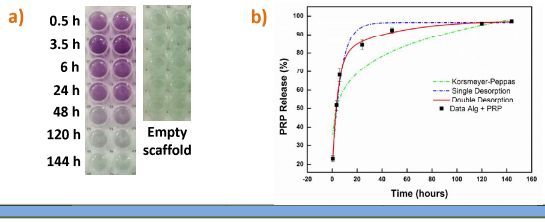
Scaffolds were realized by a 3D-extrusion bioprinter, starting from a nanofibrillated cellulose and alginate ink mixed with PRP (2×10^6 platelets/mL). The realized constructs were then cross-linked in a $CaCl_2$ solution (50mM) subsequently to the printing, thus obtaining freestanding structures.



2. Protein release kinetics

PRP-derived growth factor release through the constructs was characterized prior to encapsulate cells by performing BCA assay (a). The gradual release of growth factors from platelets is achieved during culture time, thus obtaining a beneficial micro-environment for cell functions and their interactions.

Experimental data were analyzed by two stage desorption model (b).

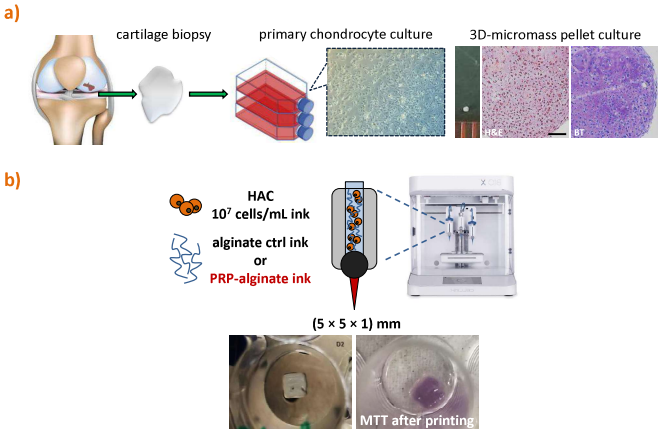


3. 3D-bioprinting of Human Articular Chondrocytes

Primary Human Articular Chondrocytes (hACs) were extracted by serial enzymatic digestions from cartilage tissue biopsies and then they were checked for their chondrogenic differentiation potential by micromass pellet culture *in vitro* (a).

Subsequently, 10^7 hACs/mL were encapsulated in PRP-alginate ink or alginate control ink and were printed (b).

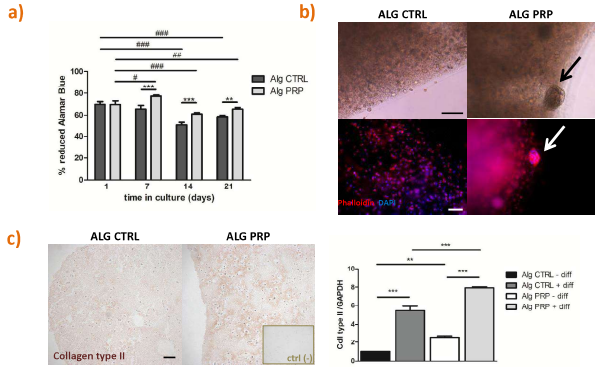
MTT staining after printing shows an homogeneous distribution of viable cells inside 3D-printed constructs.



4. *In vitro* chondrogenic culture

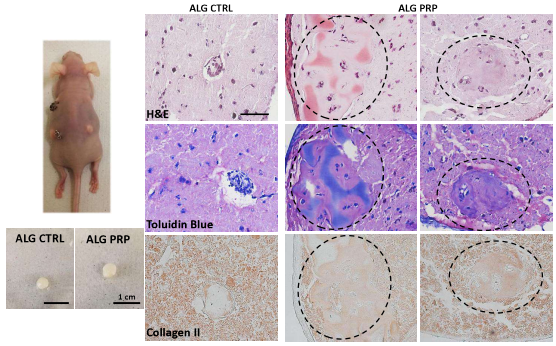
Chondrocyte-laden scaffolds were cultured in chondrogenic medium (containing TGF-β) for 3 weeks *in vitro*.

Embedding of PRP in the printed constructs supports the chondrocyte viability in the scaffolds in comparison to control constructs realized without adding of biological factors (a). Furthermore, chondrocytes inside the PRP-scaffolds start to aggregate together, which probably means a better chondrogenic differentiation than controls (b). In fact collagen type II expression, a chondrogenic marker, increases in PRP-samples compared to alginate ones (c).



5. *In vivo* chondrogenesis

The ability to sustain the *in vivo* chondrogenesis was checked by subcutaneous implantation of the printed constructs in nude mice for 1 month followed by histological analysis of those recovered. The *in vivo* test demonstrates cellular production of a glycosaminoglycan-positive matrix and organization in a hyaline-like cartilage tissue more consistent in PRP groups than control ones without platelet derivatives.



Conclusions

3D bioprinting allows to realize custom-made and autologous cartilage-like constructs available in the injury treatment or even surgery. The introduction of PRP improves the biological function of a standard printable ink and it offers the opportunity for the controlled and localized delivery of growth factors with the aim to stimulate cartilage regeneration and survival.

Acknowledgements

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[2] Murphy SV, Atala A Nat. Biotechnol. 2014; 32(8): 773