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Development of scaffolds based on natural materials for tendon tissue regeneration

Ruiz Alonso Sandra[1,2,3], Lafuente Merchan Markel[1,2,3], Gallego Idoia[1,2,3], Saenz Del Burgo Laura[1,2,3], Pedraz Jose Luis[1,2,3]

[1] NanoBiocel Group, Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain
[2] Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Vitoria-Gasteiz, Spain
[3] Bioaraba Health Research Institute, NanoBioCel Research Group, E-01009 Vitoria-Gasteiz, Spain
*Correspondencia: sandra.ruiz@ehu.eus

1. Introduction

Tendon injuries represent a clinical burden on health systems globally [1]. 3D bioprinting could help for the development of scaffolds whose characteristics resemble those of the native tendon [2].

In this study, an ink based on natural materials was developed for tendon tissue regeneration. That ink was characterized and used to print scaffolds by 3D printing. Swelling and degradation rate assays were performed to predict the possible behaviour of the scaffolds in vivo. Finally, tenocytes were incorporated into the inks and 3D bioprinted. The viability and metabolic activity of tenocytes in the scaffold were analyzed.

2. Materials and methods

2.1. Development and characterization of the ink for 3D printing

Hyaluronic acid, alginate, fibrinogen and gelatine were mixed and dissolved in DMEM culture medium to obtain the ink. Its characterization was made by two different rheological tests in the Rheometer AR1000 (TA instruments, New Castle, USA). For the oscillation test a frequency ramp was performed (-0,1 Hz to 100 Hz). For the rotational test a shear rate sweep was performed ($0.1s^{-1}$ to $100s^{-1}$/$100s^{-1}$ to $0.1s^{1}$).

2.2. 3D printing of scaffolds

2.2.1. 3D printing

The developed ink was used to print scaffolds using a BIO X 3D printer (CELLINK, Gothenburg, Sweden).

2.2.2. Swelling capacity of the scaffolds

Scaffolds were lyophilized and weighed. Then, they were immersed in PBS and kept for up to 3 days at 37 °C. The wet scaffolds were reweighed.

2.2.3. Degradation rate

Scaffolds were weighed at different times after printing, thereby controlling their weight loss after incubation in culture medium at 37ºC for several weeks.

2.3. 3D bioprinting of scaffolds

Mouse tenocytes were incorporated into the developed ink (5·106 cel/ml). Bioinks were used to obtain scaffolds by 3D bioprinting.

2.3.1. Cell viability assay

To analyse the cell viability a Live/Dead test was performed. Scaffolds were then observed on a Nikon Eclipse TE2000-S confocal microscope.

2.3.2. Bioactivity assay

Cell metabolic activity was studied using a CCK8 assay. Scaffolds were incubated with the CCK8

reagent for 4 hours at 37 ºC and subsequently the absorbance measurement was carried out in an Infinite M200 Tecan plate reader, at a wavelength of 450 nm.

3. Results and Discussion

The rotational test allowed establishing that the ink had non-Newtonian behaviour. Its shear thinning behaviour maked it ideal for 3D printing. The initial viscosity at low shear rates was 1020 Pa/s. This ink was not very thixotropic, since after applying and removing the force, the initial viscosity was not recovered. The oscillatory test made it possible to establish that the elastic modulus (G') of the ink was greater than the viscous modulus (G'') (elastic solid-like behaviour) (Fig. 1.).

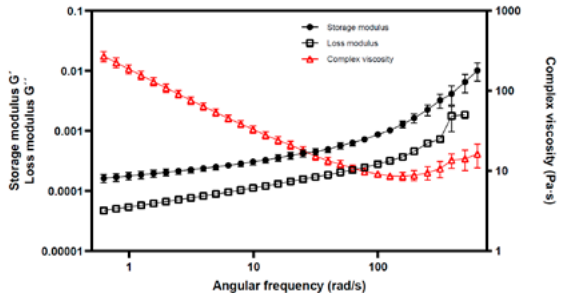
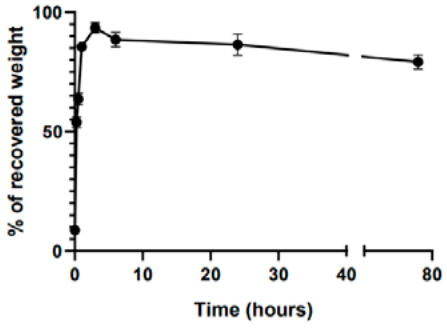


Fig. 1. Oscillation test.

As indicated by the rheological studies, the ink could be properly printed. The pressure and speed selected were 40 kPa and 7 mm/s respectively. The obtained scaffolds had dimensions very similar to those of the CAD design.

The swelling test made it possible to establish that after one hour, the lyophilized scaffold had practically recovered its initial weight (93 %) (Fig. 2A). In the degradation assay, it was observed that the highest percentage of weight loss occurred during the first two days. From day 3 to day 35, there was a very slight gradual degradation (Fig. 2B).

A)



B)

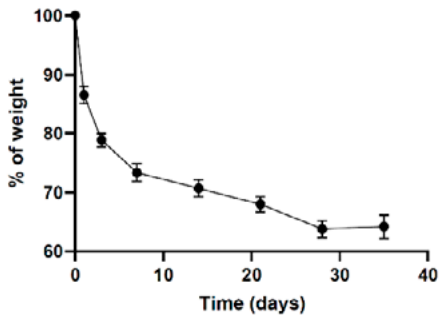
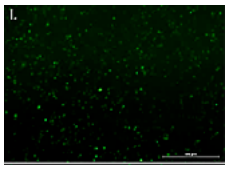


Fig. 2. A) Swelling test. **B)** Degradation test.

After the incorporation of the tenocytes in the ink, the viability and activity of the cells within the scaffolds were analyzed. The viability of the cells at different times was good (no cell death was observed) (Fig. 3B). This result was related to the one obtained for cellular activity in which an increase in activity could be seen over time (Fig. 3A).

A)



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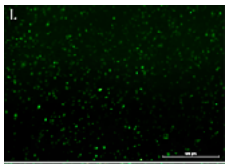

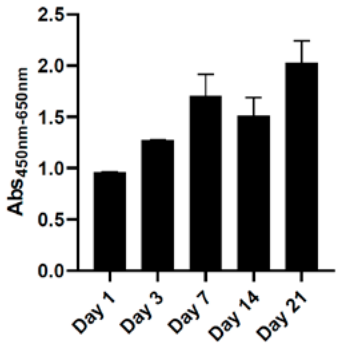

Fig. 3. A) Assay of metabolic activity. **B)** Cell viability of tenocytes at day 1 (I) and day 21 (II). Scale Bar=500 µm

4. Conclusions

The ink based on hyaluronic acid, alginate, gelatin and fibrinogen presented adequate rheological behaviour. In addition, the swelling values indicated a good rehydration capacity and the

degradation values indicated a maintenance of the scaffold structure for the appropriate time. The viabilities and cellular functionality showed that the scaffold was biocompatible. On the one hand, these good results suggest that the developed ink is optimal for its use in 3D bioprinting technology applied to tendon regeneration. On the other hand, the developed scaffolds could have a positive effect on the regeneration of damaged tendons. In future studies, the effect of these scaffolds in vivo will be analyzed.

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