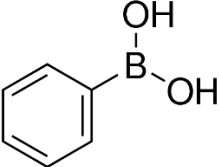
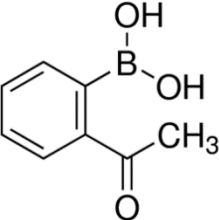
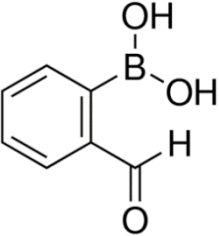
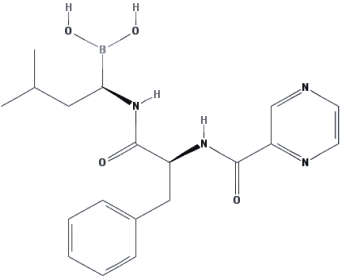


# Exploring functionalized magnetic hydrogel polyvinyl alcohol and chitosan electrospun nanofibers

Mónica Guerra<sup>1</sup>, Fábio F.F. Garrudo<sup>2</sup>, Célia Faustino<sup>1,3,4</sup>, M. Emilia Rosa<sup>5</sup> and Maria H. Ribeiro<sup>1,3,4\*</sup>

Table S1 describes the boronic acids tested in this work.

**Table S1.** Boronic acids used in the crosslinking process.

| Nomenclature IUPAC   | Abbreviation | Chemical structure   | Concentration tested (% m/v) |
|--|--------------|--|------------------------------|
| Phenylboronic acid   | PBA          |    | 1                            |
| 2-Acetyl-phenylboronic acid  | aPBA         |   | 0.5                          |
| 2-formyl-phenylboronic acid  | fPBA         |  | 0.5                          |
| [(1R)-3-Methyl-1-[[[(2S)-3-phenyl-2-(pyrazine-2-aminocarbonyl)propanoyl]amino]butyl]boronic acid | BTZ          |  | 0.5                          |

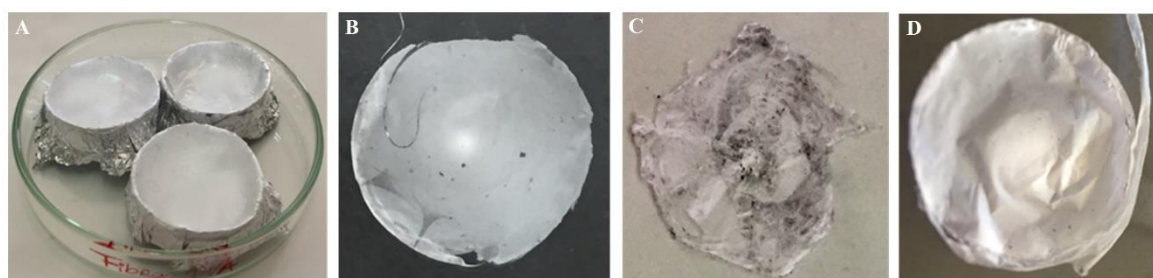
IUPAC nomenclature Abbreviation Chemical structure Tested concentration (% m/v)

**Table S2.** CCD matrix delineation of the effect of pH and temperature on releasing Lys from the nanofiber.

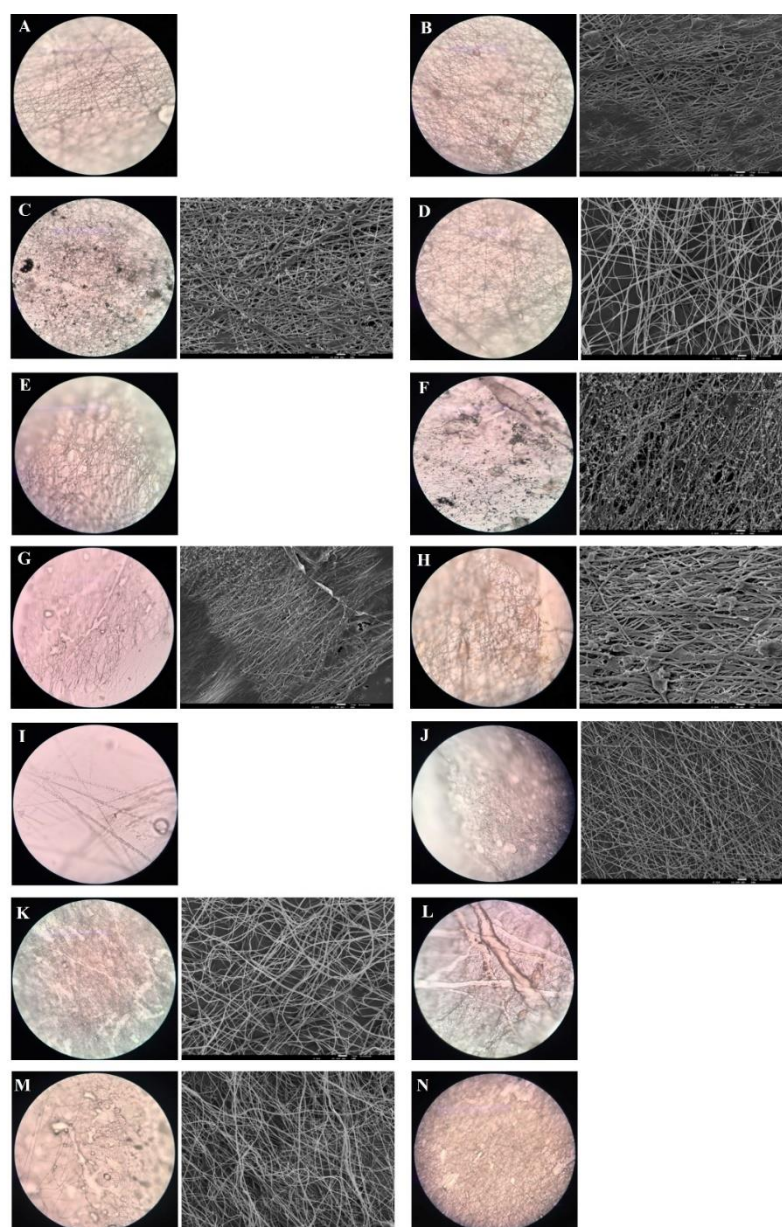
| Assay | Coded values       |                    |                  |
|-------|--------------------|--------------------|------------------|
|       | pH                 | Temperature (°C)   |                  |
| 1     | 7.60 (+1)          | 23.5 (-1)          | Factorial points |
| 2     | 6.40 (-1)          | 23.5 (-1)          |                  |
| 3     | 7.60 (+1)          | 42.5 (+1)          |                  |
| 4     | 6.40 (-1)          | 42.5 (+1)          |                  |
| 5     | 6.15 (- $\alpha$ ) | 36.5 (0)           | Axial points     |
| 6     | 7.85 (+ $\alpha$ ) | 36.5 (0)           |                  |
| 7     | 7.0 (0)            | 23.5 (- $\alpha$ ) |                  |
| 8     | 7.0 (0)            | 50.0 (+ $\alpha$ ) |                  |
| 9     | 7.0 (0)            | 36.5 (0)           | Central points   |
| 10    | 7.0 (0)            | 36.5 (0)           |                  |
| 11    | 7.0 (0)            | 36.5 (0)           |                  |

**Table S3.** CCD matrix design of the effect of blending Lys and boronic acids (PBA, aPBA, fPBA and BTZ) on the cell viability of Caco-2.

| Assay | Coded values                        |                                     |                                     |
|-------|-------------------------------------|-------------------------------------|-------------------------------------|
|       | Lys (mg/mL)                         | PBA, aPBA, fPBA (mg/mL)             | BTZ (mg/mL)                         |
| 1     | 2.00 (+1)                           | $9.76 \times 10^{-3}$ (-1)          | $4.88 \times 10^{-3}$ (-1)          |
| 2     | $9.76 \times 10^{-4}$ (-1)          | $9.76 \times 10^{-3}$ (-1)          | $4.88 \times 10^{-3}$ (-1)          |
| 3     | 2.00 (+1)                           | 20.00 (+1)                          | 10.00 (+1)                          |
| 4     | $9.76 \times 10^{-4}$ (-1)          | 20.00 (+1)                          | 10.00 (+1)                          |
| 5     | $9.76 \times 10^{-4}$ (- $\alpha$ ) | 0.63 (0)                            | 0.31 (0)                            |
| 6     | 1.44 (+ $\alpha$ )                  | 0.63 (0)                            | 0.31 (0)                            |
| 7     | 0.03 (0)                            | $9.76 \times 10^{-3}$ (- $\alpha$ ) | $4.88 \times 10^{-3}$ (- $\alpha$ ) |
| 8     | 0.03 (0)                            | 14.76 (+ $\alpha$ )                 | 7.38 (+ $\alpha$ )                  |
| 9     | 0.03 (0)                            | 0.63 (0)                            | 0.31 (0)                            |
| 10    | 0.03 (0)                            | 0.63 (0)                            | 0.31 (0)                            |
| 11    | 0.03 (0)                            | 0.63 (0)                            | 0.31 (0)                            |



**Figure S1.** Nanofibers. (A) PVA; (B) PVA/CS 70:30+Lys; (C) PVA+Lys+PBA+IONPs; (D) PVA/CS 90:10.



**Figure S2.** Optical microscopy (400x magnification) and electron microscopy (500x magnification) images of the nanofibers of: PVA (**A**); PVA+PBA (**B**); PVA+PBA+Lys (**C**); PVA+Lys (**D**); PVA+Lys+PBA (**E**); PVA+Lys+PBA+IONPs (**F**); PVA+Lys+aPBA (**G**); PVA+Lys+fPBA (**H**); PVA+Lys+BTZ (**I**); PVA/CS 90:10 (**J**); PVA/CS 90:10+Lys (**K**); PVA/CS 90:10+Lys+PBA (**L**); PVA/CS 70:30 (**M**); PVA/CS 70:30+Lys (**N**).

Table S4 showed the different concentrations of lysozyme (Lys), boronic acids and IONPs nanoparticles tested on the Caco-2 cell viability

**Table S4.** Initial concentrations used in the Caco-2 cell viability assay.

| Dilution series | Lys<br>(mg/mL) | [IONPs] (mg/mL) | Boronic acids (mg/mL) |         |
|-----------------|----------------|-----------------|-----------------------|---------|
|                 |                |                 | PBA, aPBA, fPBA       | BTZ     |
| <b>1:1</b>      | <b>1</b>       | 2               | 10                    | 5       |
| 1:2             | 0.5            | 1               | 5                     | 2.5     |
| 1:4             | 0.25           | 0.5             | 2.5                   | 1.25    |
| 1:8             | 0.125          | 0.25            | 1.25                  | 0.625   |
| 1:16            | 0.0625         | 0.125           | 0.625                 | 0.313   |
| 1:32            | 0.0313         | 0.0625          | 0.313                 | 0.156   |
| 1:64            | 0.0156         | 0.0313          | 0.156                 | 0.0781  |
| 1:128           | 0.00781        | 0.0156          | 0.0781                | 0.0391  |
| 1:256           | 0.00391        | 0.00781         | 0.0391                | 0.0195  |
| 1:512           | 0.00195        | 0.00391         | 0.0195                | 0.00977 |
| 1:102           | 0.000977       | 0.00195         | 0.00977               | 0.00488 |
| 1:205           | 0.000488       | 0.000977        | 0.00488               | 0.00244 |