Data Article

Data of a stiffness softening mechanism effect on proliferation and differentiation of a human bone marrow derived mesenchymal stem cell line towards the chondrogenic and osteogenic lineages

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A B S T R A C T

This article contains data related to the research article entitled “Stiffness memory of indirectly 3D-printed elastomer nanohybrid regulates chondrogenesis and osteogenesis of human mesenchymal stem cells” [1] (Wu et al., 2018).

Cells respond to the local microenvironment in a context dependent fashion and a continuous challenge is to provide a living construct that can adapt to the viscoelasticity changes of surrounding tissues. Several materials are attractive candidates to be used in tissue engineering, but conventional manufactured scaffolds are primarily static models with well-defined and stable stiffness that lack the dynamic biological nature required to undergo changes in substrate elasticity decisive in several cellular processes key during tissue

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development and wound healing. A family of poly(urea-urethane) (PUU) elastomeric nanohybrid scaffolds (PUU-POSS) with thermo-responsive mechanical properties that soften by reverse self-assembling at body temperature had been developed through a 3D thermal induced phase transition process (3D-TIPS) at various thermal conditions: cryo-coagulation (CC), cryo-coagulation and heating (CC + H) and room temperature coagulation and heating (RTC + H). The stiffness relaxation and stiffness softening of these scaffolds suggest regulatory effects in proliferation and differentiation of human bone-marrow derived mesenchymal stem cells (hBM-MSCs) towards the chondrogenic and osteogenic lineages.

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### Specifications table

| Subject area                        | Chemistry, biology |
|-------------------------------------|--------------------|
| More specific subject area          | Biomaterials       |
| Type of data                        | Tables, figures    |
| How data was acquired               | Static compression and tensile mechanical testing (Instron 5655), Dynamic mechanical compression (ElectroForce Biodynamic® Test Instrument 5160), Mercury intrusion porosimeter (PoreMaster 60GT Quantachrome), Immunohistochemistry, Element detection (EDX, EDAX Inc.) |
| Data format                         | Analyzed           |
| Experimental factors                | Compression and tensile mechanical properties in static mode were evaluated with Instron; dynamic compression testing with an Electro-Force bioreactor. The hierarchical porous structure of the scaffolds was analyzed via mercury intrusion porosimeter. Chondrogenic differentiation was studied via Hematoxylin and Eosin, Alcian Blue and Collagen II staining; osteogenic differentiation was studied via Hematoxylin and Eosin, Alizarin Red and Collagen I staining. Energy-dispersive X-ray analysis (EDX) was carried out for elemental mapping analysis. |
| Experimental features               | Physico-mechanical characterization, histology and immunohistochemistry |
| Data source location                | N/A                |
| Data accessibility                  | Within this article |

### Value of the data

- Data presented here provides optimized conditions for the assessment of mesenchymal stem cell differentiation on stiffness softening scaffolds.
- Compression mechanical testing along with histological assessment was sensitive to elucidating how stiffness softening affects stem cell differentiation.

1. **Data**

Fig. 1 depicts cell expansion and differentiation of human bone-marrow derived mesenchymal stem cells (hBM-MSCs) on 3D-TIPS PUU-POSS scaffolds exhibiting stiffness softening. Table 1 shows
the effect of the infill density (i.e. 3D printing) and the various 3D-TIPS thermal conditions (i.e. CC, CC + H and RTC + H) on the mechanical properties of the scaffolds. Table 2 demonstrates the isothermal stiffness softening behaviour of 50% infill density scaffolds after a 28-day period incubation in vitro at body temperature (37°C), with all scaffold groups reaching their intrinsic elasticity (i.e. 'stiffness memory' concept). Table 3 shows viscoelastic behaviours of 50% infill density scaffolds during dynamic compression testing, all reaching their intrinsic elasticity. Fig. 2 and Table 4 demonstrate the hierarchical micro-/nano- porous structure of the various scaffold groups. Figs. 3 and 5 show hist-

**Table 1**

| Scaffold  | Infill density, % | Scaffold density, \( d_s \), kg/m \(^3\) | Total porosity, 100% | Compression strength, MPa | Compression modulus, MPa |
|-----------|-------------------|------------------------------------------|----------------------|-------------------------|-------------------------|
| CC        | 80                | 44 ± 3                                   | 96.2 ± 0.3           | 0.54 ± 0.02             | 0.82 ± 0.03             |
| CC        | 70                | 40 ± 3                                   | 96.5 ± 0.3           | 0.48 ± 0.01             | 0.75 ± 0.01             |
| CC        | 60                | 37 ± 5                                   | 96.8 ± 0.4           | 0.34 ± 0.01             | 0.63 ± 0.02             |
| CC        | 50                | 36 ± 4                                   | 96.9 ± 0.4           | 0.33 ± 0.03             | 0.48 ± 0.08             |
| CC        | 40                | 30 ± 6                                   | 97.4 ± 0.5           | 0.17 ± 0.04             | 0.39 ± 0.03             |
| CC        | 30                | 27 ± 3                                   | 97.7 ± 0.3           | 0.10 ± 0.02             | 0.25 ± 0.02             |
| CC + H    | 80                | 56 ± 8                                   | 95.1 ± 0.7           | 0.38 ± 0.01             | 0.56 ± 0.01             |
| CC + H    | 70                | 51 ± 4                                   | 95.5 ± 0.3           | 0.34 ± 0.04             | 0.41 ± 0.02             |
| CC + H    | 60                | 49 ± 3                                   | 95.8 ± 0.3           | 0.26 ± 0.02             | 0.37 ± 0.03             |
| CC + H    | 50                | 45 ± 5                                   | 96.1 ± 0.5           | 0.21 ± 0.01             | 0.27 ± 0.03             |
| CC + H    | 40                | 41 ± 4                                   | 96.5 ± 0.3           | 0.11 ± 0.01             | 0.20 ± 0.02             |
| CC + H    | 30                | 37 ± 2                                   | 96.8 ± 0.2           | 0.13 ± 0.01             | 0.12 ± 0.01             |
| RTC + H   | 80                | 48 ± 10                                  | 95.8 ± 0.8           | 0.35 ± 0.01             | 0.28 ± 0.01             |
| RTC + H   | 70                | 43 ± 4                                   | 96.2 ± 0.4           | 0.25 ± 0.02             | 0.26 ± 0.02             |
| RTC + H   | 60                | 39 ± 5                                   | 96.6 ± 0.4           | 0.22 ± 0.01             | 0.22 ± 0.01             |
| RTC + H   | 50                | 38 ± 3                                   | 96.7 ± 0.3           | 0.17 ± 0.02             | 0.15 ± 0.03             |
| RTC + H   | 40                | 33 ± 5                                   | 97.1 ± 0.4           | 0.12 ± 0.01             | 0.13 ± 0.01             |
| RTC + H   | 30                | 29 ± 3                                   | 97.5 ± 0.3           | 0.10 ± 0.01             | 0.10 ± 0.01             |
Table 2
Physical and mechanical properties of 3D-TIPS PUU-POSS scaffolds (50% infill density) before and after incubation at body temperature (37°C) for 28 days.

| 3D-TIPS scaffold, 50% infill | Scaffold density, kg/m³ | Total porosity, 100% | Young’s modulus, MPa (Tensile) | Ultimate tensile strength, MPa (Tensile) | Ultimate tensile strain, % (Tensile) | Toughness, J m⁻³ × 10⁴ (Tensile) | Compression strength@25%, MPa | Compression modulus@25%, MPa |
|------------------------------|------------------------|----------------------|-------------------------------|----------------------------------------|-----------------------------------|---------------------------------|-------------------------------|-----------------------------|
| 50CC Day 0                   | 36 ± 4                 | 96.9 ± 0.4           | 0.98 ± 0.14                   | 1.33 ± 0.09                            | 179 ± 8                           | 137 ± 22                        | 0.33 ± 0.02                   | 0.51 ± 0.08                  |
| Day 28                       | 29 ± 4                 | 97.4 ± 0.3           | 0.45 ± 0.08                   | 0.77 ± 0.15                            | 230 ± 13                          | 115 ± 20                        | 0.18 ± 0.03                   | 0.16 ± 0.01                  |
| 50CC + H Day 0               | 45 ± 5                 | 96.1 ± 0.5           | 0.53 ± 0.02                   | 0.76 ± 0.05                            | 236 ± 19                          | 113 ± 27                        | 0.22 ± 0.04                   | 0.27 ± 0.03                  |
| Day 28                       | 39 ± 5                 | 96.7 ± 0.4           | 0.39 ± 0.09                   | 0.72 ± 0.12                            | 240 ± 18                          | 110 ± 14                        | 0.17 ± 0.02                   | 0.13 ± 0.01                  |
| 50RTC + H Day 0              | 38 ± 3                 | 96.7 ± 0.3           | 0.44 ± 0.06                   | 0.67 ± 0.03                            | 146 ± 15                          | 146 ± 12                        | 0.17 ± 0.05                   | 0.15 ± 0.01                  |
| Day 28                       | 32 ± 3                 | 97.2 ± 0.3           | 0.42 ± 0.08                   | 0.65 ± 0.06                            | 149 ± 19                          | 146 ± 20                        | 0.17 ± 0.02                   | 0.12 ± 0.01                  |
Table 3
Hysteresis values (i.e. energy loss) of the various scaffolds (50% infill density) during tensile and compression cyclic loading at day 0 and after incubation for 28 days at 37°C.

| Type of test | Day   | Hysteresis energy (J/m^3) | 50CC | 50CC + H | 50RTC + H |
|--------------|-------|---------------------------|------|----------|-----------|
| **Tensile**  | **D0** | 0–200 cycles              | 160 ± 11 | 21 ± 8  | 15 ± 7   |
|              |       | 1000–1200 cycles          | 133 ± 1 | 18 ± 2  | 13 ± 2   |
|              |       | 10,000–10,200 cycles      | 24 ± 8  | 14 ± 3  | 13 ± 4   |
|              |       | 200,000–200,100 cycles    | 15 ± 5  | 8 ± 3   | 11 ± 4   |
|              | **D28** | 0–200 cycles              | 31 ± 6  | 17 ± 4  | 12 ± 4   |
|              |       | 1000–1200 cycles          | 17 ± 6  | 13 ± 5  | 10 ± 2   |
|              |       | 10,000–10,200 cycles      | 12 ± 5  | 10 ± 4  | 9 ± 3    |
|              |       | 200,000–200,200 cycles    | 10 ± 4  | 10 ± 4  | 9 ± 3    |
| **Compression** | **D0** | 0–200 cycles              | 274 ± 7  | 125 ± 10 | 12 ± 4   |
|              |       | 1000–1200 cycles          | 124 ± 9  | 91 ± 10 | 14 ± 4   |
|              |       | 10,000–10,200 cycles      | 101 ± 10 | 81 ± 4  | 13 ± 4   |
|              |       | 100,000–100,200 cycles    | 90 ± 8  | 80 ± 4  | 12 ± 4   |
|              |       | 200,000–200,100 cycles    | 60 ± 5  | 52 ± 3  | 10 ± 4   |
|              | **D28** | 0–200 cycles              | 63 ± 5  | 56 ± 8  | 8 ± 3    |
|              |       | 1000–1200 cycles          | 43 ± 5  | 35 ± 9  | 10 ± 5   |
|              |       | 10,000–10,200 cycles      | 31 ± 5  | 23 ± 7  | 10 ± 4   |
|              |       | 100,000–100,200 cycles    | 13 ± 5  | 15 ± 4  | 8 ± 4    |
|              |       | 200,000–200,100 cycles    | 10 ± 4  | 9 ± 4   | 8 ± 3    |

Fig. 2. Porosity analysis of 50% infill density scaffolds. Mercury porosimeter measurements in terms of pore size and pore size distribution [2];

tological sectioning demonstrating chondrogenic and osteogenic differentiation, respectively, on the various scaffolds. Fig. 4 and Tables 5 and 6 show elemental mapping analysis after chondrogenic and osteogenic differentiation on the various scaffolds.

1.1. Cell expansion and differentiation of hBM-MSCs on 3D-TIPS PUU-POSS scaffolds

See Fig. 1.

1.2. Physico-mechanical characterization and ‘stiffness memory’ of 3D-TIPS PUU-POSS scaffolds

See Tables 1-4 and Fig. 2.

1.3. Chondrogenic and osteogenic evaluation

See Figs. 3-5 and Tables 5 and 6.
Table 4
Pore size and pore size distribution of 50% infill density scaffolds [2].

| Scaffold  | Pore diameter, nm | Pore volume, cm³/g | Relative pore volume, % | Surface area, m²/g | Relative surface area, % |
|-----------|-------------------|--------------------|-------------------------|-------------------|-------------------------|
| 50CC      | 456,882–1000      | 29.75              | 58.46                   | 1.55              | 2.65                    |
|           | 1000–100          | 11.25              | 22.10                   | 48.67             | 83.16                   |
|           | 100–3             | 9.89               | 19.44                   | 8.30              | 14.19                   |
| Total     |                   | 50.89              | 100                     | 58.52             | 100                     |
| 50CC + H  | 439,998–1000      | 31.31              | 75.75                   | 1.47              | 6                       |
|           | 1000–100          | 6.57               | 15.89                   | 18.82             | 76.84                   |
|           | 100–3             | 3.45               | 8.36                    | 4.2               | 17.16                   |
| Total     |                   | 41.33              | 100                     | 24.49             | 100                     |
| 50RTC + H | 387,810–1000      | 48.64              | 95.16                   | 2.68              | 58.51                   |
|           | 1000–100          | 0                  | 0                       | 0                 | 0                       |
|           | 100–3             | 2.47               | 4.84                    | 1.9               | 41.49                   |
| Total     |                   | 51.11              | 100                     | 4.58              | 100                     |

Fig. 3. Chondrogenic differentiation on 50% infill density scaffolds. (A-I) Histological analysis of chondrogenic differentiation at week 4: in cross-section for (A, D, G) 50CC, (B, E, H) 50CC + H, and (C, F, I) 50RTC + H. Stained with Hematoxylin and Eosin (H&E), Alcian Blue (A-Blue) and Collagen II (COL2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).
2. Experimental design, materials and methods

2.1. 3D-TIPS PUU-POSS scaffold manufacturing

3D-TIPS PUU-POSS scaffolds at different thermal conditions (Cyo-coagulation, CC; cryo-coagulation and heating, CC + H; and room temperature coagulation and heating, RTC + H) were manufactured by a 3D confined thermal induced phase separation process (3D-TIPS) based on self-assembly, phase transition and phase separation of the polymeric solution at controlled temperatures as described in [1,2].

2.2. Cell expansion and differentiation

A human bone marrow derived mesenchymal stem cell line was expanded, seeded and differentiated (Table S1-S2) on 3D-TIPS PUU-POSS scaffolds with stiffness softening as described in [1].
Fig. 5. Osteogenic differentiation on 50% infill density scaffolds. (A-I) Histological analysis of osteogenic differentiation at week 4: in cross-section for (A, D, G) 50CC, (B, E, H) 50CC +H, and (C, F, I) 50RTC +H. Stained with H&E, Alizarin red, and Collagen I (COL1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).
2.3. Physico-mechanical characterization of the scaffolds prior to cell seeding

Static mechanical testing of the scaffolds under tensile and compression mode, for different infill densities, before and after incubation over 28 days at body temperature in vitro (37°C), was performed with an Instron 5655 tester as described previously [1].

A mercury intrusion porosimeter (PoreMaster 60GT, Quantachrome, UK) was used to characterise the pore structure including the pore size, pore volume, size distribution and surface area of freeze-dried scaffolds (50% infill density).

2.4. Chondrogenic and osteogenic assessment

Element detection on cell-laden 50% infill density scaffolds after differentiation was quantified via Energy-dispersive X-ray (EDX) analysis as described in [1].

Histological section and staining of the scaffolds (50% infill density) was performed after chondrogenic and osteogenic differentiation as previously described [1].

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Transparency document. Supporting information

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.09.068.

Reference

[1] L. Wu, A. Magaz, T. Wang, C. Liu, A. Darbyshire, M. Loizidou, M. Emberton, M. Birchall, W. Song, Stiffness memory of 3D-printed elastomer nanohybrid regulates chondrogenesis and osteogenesis of human bone-marrow derived mesenchymal stem cells, Biomaterials (2018), http://dx.doi.org/10.1016/j.biomaterials.2018.09.013.

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