Supplementary 1: Uniaxial compression of scaffolds seeded with co-cultures

Aim of this supplementary

The aim of this supplementary is to evaluate stability of the collagen-coated carboxymethylcellulose microscaffolds (CCMs) seeded with cocultures of murine stromal OP9 and hematopoietic stem and progenitor cells (HSPC, obtained by sorting for ckit^{+}, lin-, Sca-1^{+} "KLS"). Seeded at an initial OP9:KLS ratio of 100:1, the CCMs were cultivated for 3 months to achieve steady-state cellular composition prior to mechanical and cellular stability testing against controlled uniaxial compression. This data allows to follow the fate of the co-cultures on the CCMs during controlled compression, and thus to estimate the allowable compression during injection.

Materials and Methods

Seeding and culture

The aim was to obtain densely populated CCMs to best evaluate the effect of compression from confocal images; to minimize the amount of required KLS, we seeded the scaffolds in relatively low density, but then let the KLS and OP9 reach high confluence by extended culture time.

Collagen-coated CCMs were seeded as outlined using 75,000 OP9 (GFP) and 7,500 KLS (DsRed) per mg of dry scaffold weight. After initial incubation to allow for cell adherence, the scaffolds were distributed in ultra-low adhesion plates; a 6-well plate (2mg of dry scaffold/well) and a 24 well plate (0.5mg of dry scaffold per well). Culture was performed by adding first 3mL respectively 0.5mL of conditioned medium, and then another 3mL respectively 0.5mL at day 7, followed by half-media changes every week for 3 months. We took care to avoid aspiration of the CCMs by holding the plate in a slanted position for about 30s prior to medium aspiration. This allows the CCMs to sediment and be protected from aspiration. The procedure however is expected to remove a sizeable fraction of the cellular descendance generated by *in-vitro* hematopoiesis at each medium change.

Sample preparation for uniaxial compression

Scaffolds were retrieved at 3 months of culture and pipetted into an observation chamber adapted for removal of excess liquid during the concentration process (Fig S1-1A, exploded view).

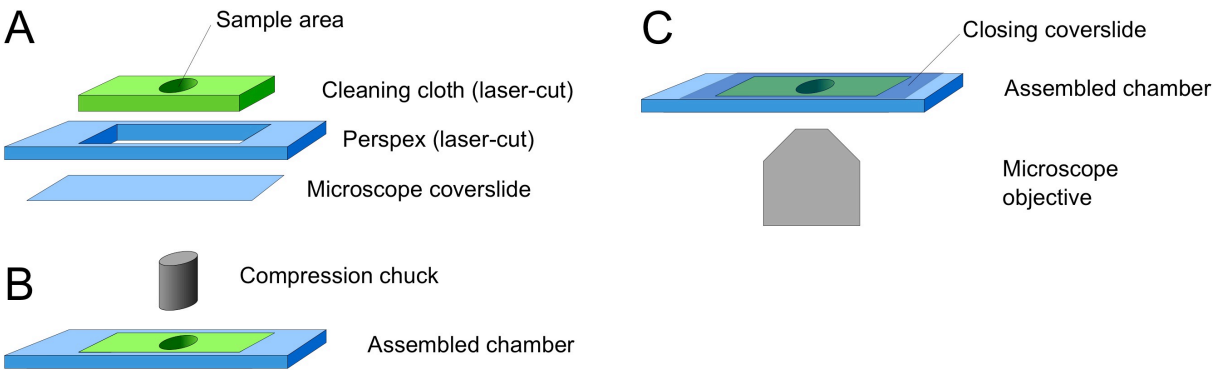

Fig. S1-1. Observation chamber adapted for uniaxial compression. A) Fabrication: The chamber is assembled from a cleaning cloth, a Perspex sheet and a microscope coverslide. The Perspex sheet is cut to the outer dimensions of a microscope slide (75mm x 25mm) with a CO_2 laser cutter, with a central observation area (30x12.5mm). It is closed on the lower side with a microscope coverslide, which is glued to the Perspex sheet with silicone glue. The assembly is completed with a cleaning cloth, cut to fit into the observation area, and with a central sample area of circular shape (8mm) diameter. B) Use in compression. Using an 8mm diameter chuck on a texture Analyzer XTPlus machine, sample in the sample area can be compressed uniaxially. Excess liquid is taken up by the cleaning cloth. C) After compression, the sample can be observed by confocal microscopy. For this purpose, the chamber can be closed reversibly by a second microscope coverslide to limit evaporation.

This chamber consisted of a laser cut Perspex sheet in the shape of a microscope slide (i.e. 75mm x 25mm), with a rectangular central observation area (30mm x 12.5mm), closed with a floor consisting of a glued coverslide (Fig. S1-1A). In addition, we placed a laser-cut cleaning cloth made from polypropylene and cellulose (Migros, Switzerland) into the central area (Fig. S1-1A). The cleaning cloth matched the dimensions of the central observation area (nominally 30mm x 12.5mm, de facto about 0.25mm smaller at each edge due to the finite laser spot size), but in addition we cut a circular hole at its center (8mm nominal diameter, real diameter estimated about 8.5mm). This circular hole serves as sample concentrator, but also as holder for uniaxial compression with a 8mm circular chuck (Fig. S1-1B). After compression, the samples can be observed by confocal microscope (Fig. S1-1C).

Prior to scaffold placement, we hydrated the cleaning cloth with the circular hole with cell culture medium (i.e. 50/50 mix of conditioned and fresh medium as described in the main text) supplemented with 50mM HEPES for use in the absence of a CO_2 enriched atmosphere. We then slowly pipetted the suspension of CCMs from the cultures into the central hole. To avoid compression during transfer, we aseptically broke open a medium aspiration pipet for this purpose. At the same time, we removed excess medium by aspiration from the cleaning cloth. Taking care to always leave sufficient medium to have the gap between the observation chamber wall and the cleaning cloth filled with medium, we avoided excessive capillary pressure on the accumulating material. In this way, we obtain an assembled biomaterial where the particles have about their natural size. By sedimentation of CCMs (unseeded) in large excess of medium, we estimated the polymer concentration at full expansion to be about 8mg/mL (see below, section “Uniaxial compression properties”). We added enough material to completely, but not excessively fill the sample hole with cell-seeded

biomaterial; we repeated the experiment 2x, once with material from the 6-well culture, once from the 24-well culture.

Uniaxial compression

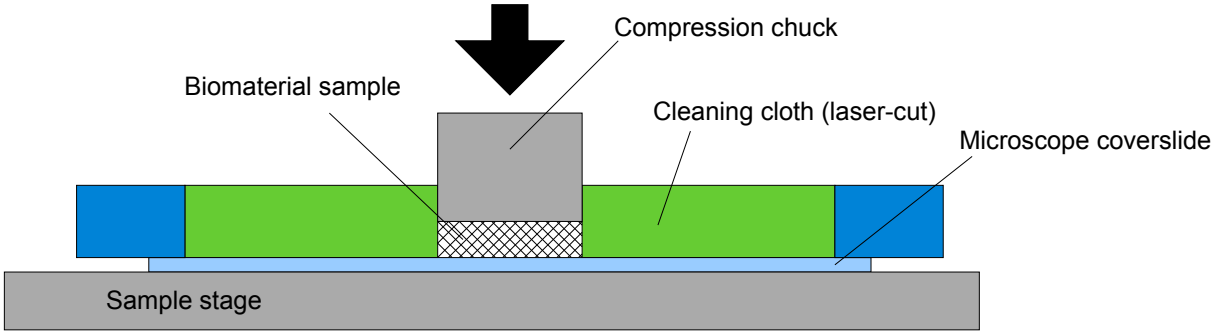

Fig. S1-2. Uniaxial sample compression. A sample is loaded into the circular sample area (Fig. S1-1, here in cross-section), defined within the cleaning cloth. The compression chamber is then placed onto the sample stage of a mechanical testing machine (TextureAnalyzer XTPlus) and then compressed by uniaxial movement of the chuck. Excess liquid squeezed out from the pore space during compression is drained by the cleaning cloth.

Fig. S1-2 shows the detailed setup used for uniaxial sample compression. After mounting of a 8mm cylindrical chuck onto a TextureAnalyzer XTPlus machine, the machine is calibrated for force gain by the standard calibration routine in the TextureExponent user interface. A microscope coverslide identical to the one used for compression chamber fabrication is then placed onto the sample stage of the TextureAnalyzer XTPlus machine, and chuck height calibration is carried out. In this way, the zero-height is defined at upper surface of the coverslide. Once the calibration routines successfully carried out, the compression chamber with previously loaded sample is placed under the chuck, giving rise to the compression setup as shown in Fig. S1-2. The chuck is then lowered to establish contact with the sample, taking care to avoid air bubbles. Then, the chuck is moved vertically until zero net force is detected; this is done by a built-in routine for this purpose available in the TextureExponent program.

From this zero-force equilibrium height, uniaxial compression is carried out. Three heights are tested: 0% compression, corresponding to no further movement of the chuck; 75% compression, corresponding to a decrease of the sample height to 25% of the original sample height above the coverslip surface; and 100% compression, corresponding to theoretical lowering of the chuck to the coverslip surface. In practice, even at 100% theoretical compression, some unknown, but minimal sample height is maintained due to mechanical compliance in the TextureAnalyzerXT Plus machine. The 0% percent control is to assess potential damage done to the cells by the contact with the chuck. All compression is carried out a speed of 0.01mm/s, to emulate quasi-static conditions.

Mechanical data acquisition and treatment

Force-distance data was acquired during the compression phase. This was done purposefully at relatively high data rate (200 samples per second). We could then lower the noise to a level compatible with straightforward curve analysis by applying a Gaussian low-pass filter (with a width, as expressed by the standard deviation parameter, of 40micrometers or equivalently 800 measurements).

Viability and cellular composition

For each of the two experiments done, we assessed viability and cellular composition initially (after chamber loading), at 0% compression (chuck zeroing), at 75% compression and at 100% compression. This implies that we followed 2 samples through the entire process of loading, chuck zeroing, and compression to 75% and then to 100%.

For viability estimation, we used both extrinsic staining by the nuclear stain Hoechst 328 and loss of intrinsic fluorescence (GFP for OP9, DsRed for KLS and descendance). We had indeed noticed on preliminary trials (100% compression vs. samples directly from culture) that Hoechst 328 strongly stains the nuclei of dead cells, and weakly are the ones of live cells and the scaffold material. Upon cell death, on the other hand, the GFP signal is lost rapidly, followed on the time scale of about 20 minutes by loss of DsRed as well.

Hence, we could obtain an estimate of the dead cell fraction as well as an indication of the composition (OP9 vs. KLS and descendance) of the remaining live cell population under different conditions by counting predominantly red, predominantly green, and predominantly blue cells on the confocal images.

In practice, we acquired 5 to 7 confocal stacks with at least 8 slices (Zeiss LSM700 microscope, Plan-Apochromat 20x with numerical aperture of 0.8, area of 640microns x 640microns, z-spacing 9.25 microns) for each sample and the 4 different conditions: A) sample loading only; B) sample loading plus chuck positioning; C) 75% compression; D) 100% compression. We repeated the experiment twice, once with a sample assembled from CCMs grown in a 6-well plate, once with a sample from the 24-well culture.

The observation chambers were kept closed with a second coverslide during transport and microscopic observation; between experiments, medium was replenished if necessary by placing a drop of medium (50/50 mix of conditioned and fresh medium with 50mM HEPES as described above) such as to keep the clefts between cleaning cloth and Perspex sheet fully filled, indicating minimal capillary pressure. This was necessary since opening and closing by sliding the closing coverslide (Fig. S1-1C) removed a small amount of medium sticking to the coverslide.

Cell population at 3 months

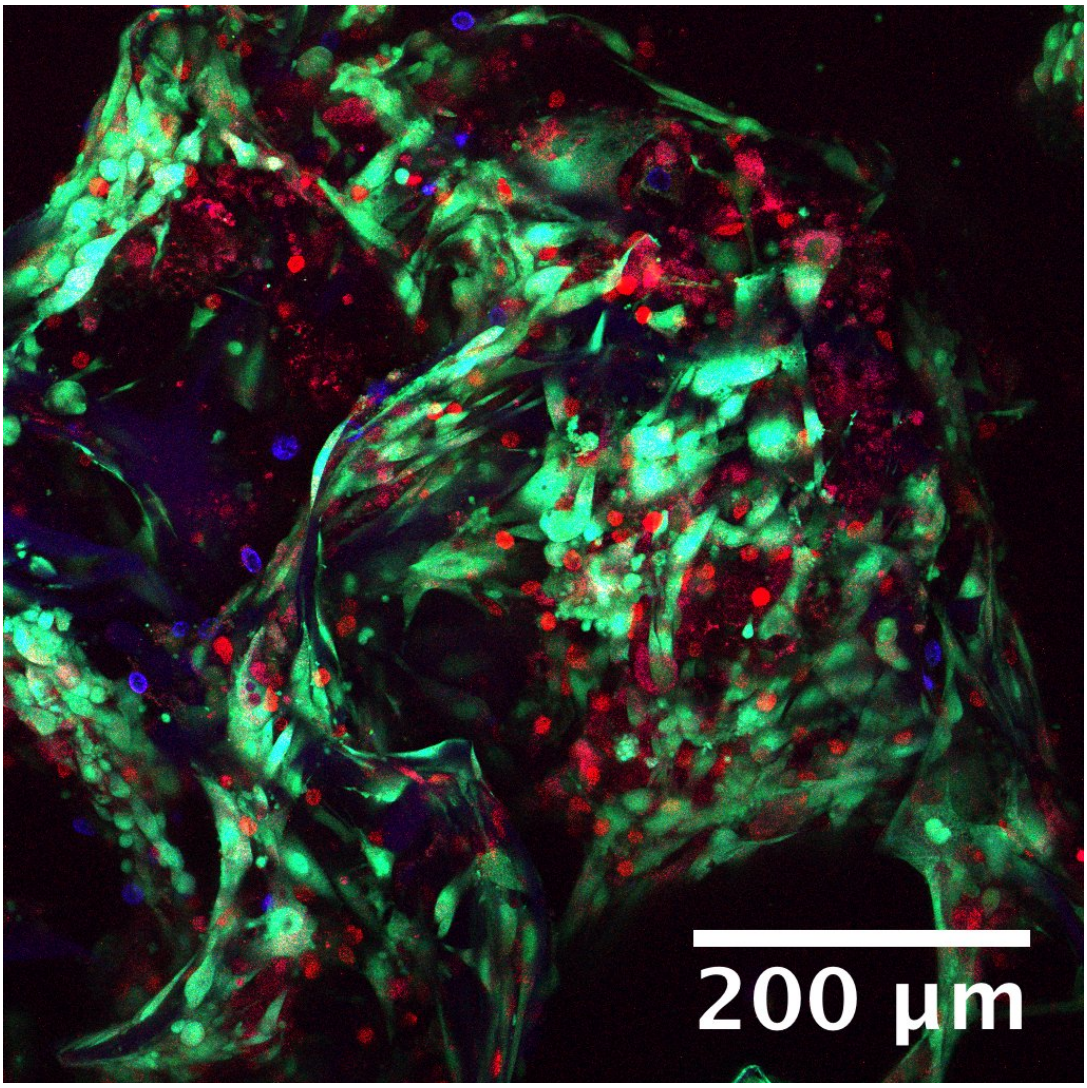

Fig. S1-3. Z-projection (maximum intensity) of a confocal stack of a co-culture of OP9 and KLS on CCMs at 3 months. Hoechst 328 was used to stain for nuclei of dead cells.

From the confocal images acquired before any contact with the compression chuck (Fig. S1-3 for an example), we could estimate the cellular population adherent to the scaffolds at 3 months. On average, we find an approximately equal amount of red (44%+/-7%, HSPC) and green cells (49%+/-8%, OP9) and a smaller fraction of dead cells (7%+/-3%).

The result suggests that on the long run, the OP9 can sustain about a 1:1 loading with firmly attached HSPC (t-test of the proportion of green vs. red cells per stack, with Moulton correction for clustering in two experiments: P=0.28, which is compatible with the null hypothesis of a 1:1 association and therefore equality of the two proportions).

We also find many cells floating freely in the wells, but due to the regular half-media changes and also the removal of most of the medium during reconstitution of a semi-solid biomaterial in the sample area of the compression chamber, we expect the quantification on the scaffolds to include nearly only cells that are firmly attached at 3 months.

Of note, in addition to well-identifiable round DsRed cells, we also find a variable proportion of DsRed that seems to be distributed in small vesicles in the cytoplasm of the corresponding OP9 cell. Although we did not investigate the phenomenon any further, this could be endo-lysosomal processing of dead DsRed positive cells.

Cell viability during compression

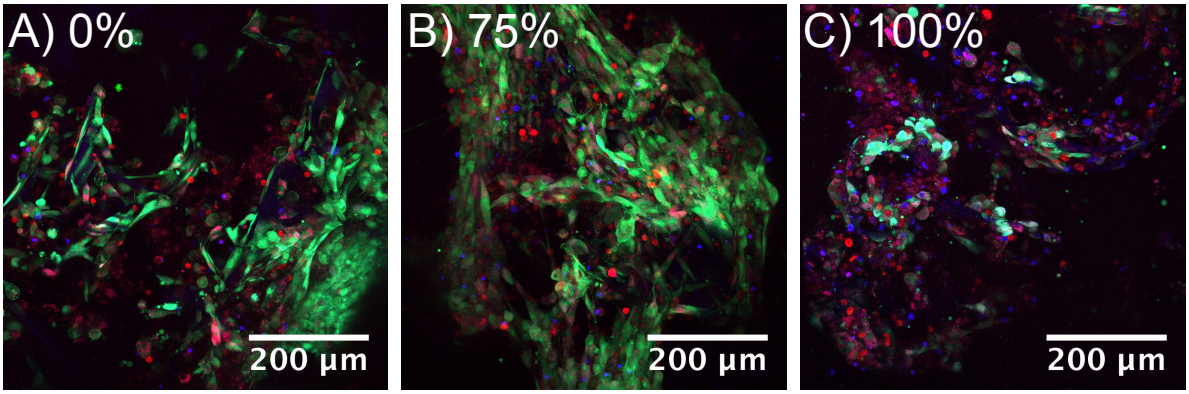

Fig. S1-4. Z-projection (maximum intensity) of a confocal stack of co-cultures of OP9 and KLS on CCMs after uniaxial compression by 0% (A), 75% (B) and 100% (C) of the original sample height. Hoechst 328 was used to stain for nuclei of dead cells.

Fig. S1-4 shows confocal images illustrating the effect of various degress of uniaxial compression (0%, 75%, 100%) on the co-cultures of OP9 (green) and KLS+ descendance (red). Fig. S1-4A and Fig. S1-4B show that qualitatively, mere contact with the chuck (Fig. S1-4A) and even transient uniaxial compression by 75% of the original sample height (Fig. S1-4B) has only minor effects on the co-cultures, whereas 100% compression kills a large fraction of the cells.

Fig. S1-5 shows a quantitative analysis of the percentage of dead cells compared to the estimated total number of cells. While no significant difference can be detected between the material simply loaded into the observation chamber (“Control: Loading” in Fig. S1-5) and contact with the compression chuck but no actual compression. We detect a significant increase in the dead cell fraction after the 75% uniaxial compression, although the co-culture still remain largely intact (raise of the dead cell fraction from 7% to 13%). Theoretically complete compression on the other hand leads to the immediate death of more than 50% of the cells.

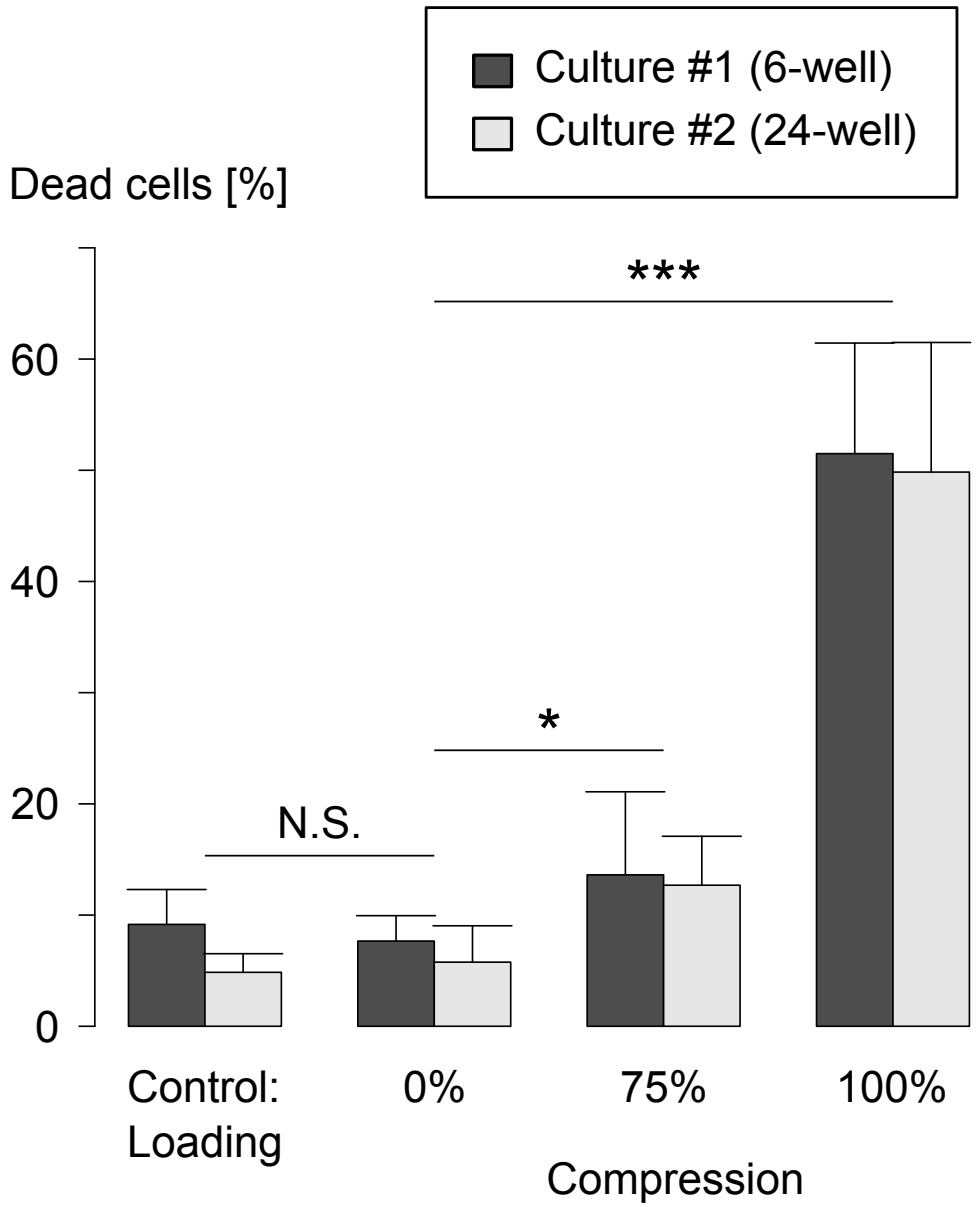

Fig. S1-5. Quantification of cell death at different levels of compression. The loading control corresponds to transfer to the compression chamber only, without contact with the chuck; this is closest to the actual culture conditions. 0% compression indicates contact and zero-force equilibration with the chuck of the uniaxial testing machine (TextureAnalyzer XTPlus), but no actual sample compression. 75% indicates a decrease in sample height by 75%, whereas 100% indicates theoretical total compression. The fraction of dead cells is the fraction of predominantly blue cells after Hoechst 328 staining, as compared to the total number of cells (predominantly blue + predominantly red + predominantly green). The compression experiments were repeated twice, once with starting material from a 6-well-plate culture, once with starting material from a 24-well-plate culture. Statistical significance was evaluated pairwise by comparison to the 0% compression condition. For this, linear regression with the experiment and condition (0% compression vs. loading control, 75% compression, 100% compression) as explanatory variables and the dead cell fraction as the outcome was used, and the P-value associated with the condition evaluated for significance.

Composition of the viable cell fraction during compression

Beyond cell viability, we also assessed the composition of the remaining viable cell population in terms of hematopoietic cells vs. OP-9 stromal cells. It could indeed conceivable be that compression, while maintaining viability of the attached cells, preferentially expels one cell type or the other. To guard against this possibility, we evaluated the fraction of red fluorescent DsRed+ cells among the total viable cell fraction at different levels of compression, and also after loading of the chamber only.

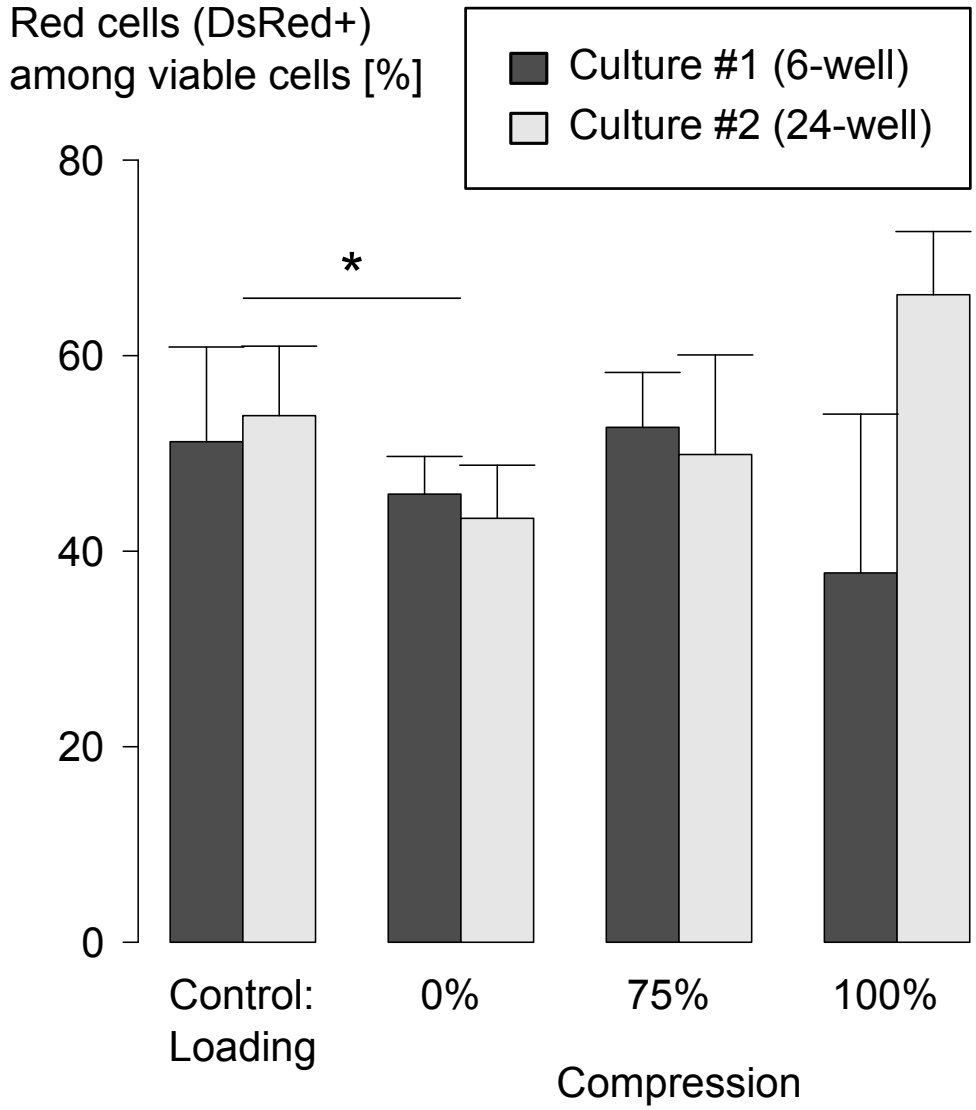

Fig. S1-6. Quantification of the composition of the remaining viable cell fraction. The DsRed+ fraction is the fraction of red fluorescent cells compared to green + red fluorescent cells; conditions are as for Figure S1-5, and evaluation based on the same images. Statistical significance was evaluated pairwise by comparison to the 0% compression condition. For this, linear regression with the experiment and condition (0% compression vs. loading control, 75% compression, 100% compression) as explanatory variables and the DsRed+ fraction as the outcome was used, and the P-value associated with the condition evaluated for significance.

Fig. S1-6 shows the results regarding the composition of the remaining viable cell fraction. This fraction is composed of distinct green (GFP+, OP9) and red (DsRed+, KLS and descendance) fluorescent cells. As outlined above, at 3 months of culture, there is a nearly 1:1 ratio of red and green cells ("Control: Loading"). We find a slight, but statistically significant decrease of the DsRed+ fraction after the chuck zero-force equilibration procedure (from 53% to 45% on average, P-value of 0.04 associated with equilibiration vs. initial loading in linear regression with both the culture and equilibration vs. initial loading as explanatory variables, Bonferroni correction for a total of three tests against the 0% compression condition). Compression by itself then has little influence on the average DsRed+ fraction, although the variability increases especially for the 100% compression, where cells are killed in entire image areas.

From the data presented in Fig. S1-6, we conclude that there is no major bias in the composition of the viable cell fraction upon compression. This result has to be interpreted within the context of a culture with regular half-media change, and dehydration during loading into the observation chamber. Indeed, we expect most of the free-floating or loosely attached cellular descendance of the hematopoietic stem and progenitor cells to be lost during culture, and at latest during chamber loading. The cells which are present after chamber loading are therefore relatively firmly adherent cells. Compression of the scaffolds with this firmly adherent cell populations seems to kill the cells located in unfavorable areas, rather than to preferentially affect one cell type or the other.

Uniaxial compression properties

The uniaxial compression experiments directly yield force-distance graphs: while the chuck slowly compresses the sample, the TextureAnalyzerXTPlus machine measures the force the sample opposes to compression.

After application of a low-pass filter to smooth the noisy, but high-bandwidth force measurements, we converted the force-distance curves to stress-strain diagrams. The stress σ is indeed calculated by relating the measured force *F* to the cross-sectional area of the chuck *A*[1] :

$$\sigma = \frac{F}{A} \qquad \text{eq. S1-1}$$

The strain ε in turn is obtained by relating the compressive chuck displacement Δh to the sample height h_0 :

$$\varepsilon = \frac{\Delta h}{h_0} \qquad \text{eq. S1-2}$$

Eq. S1-1 and eq. S1-2 correspond to the so-called « engineering »[1] stress and strain, since the original sample dimensions are used in their calculation[1].

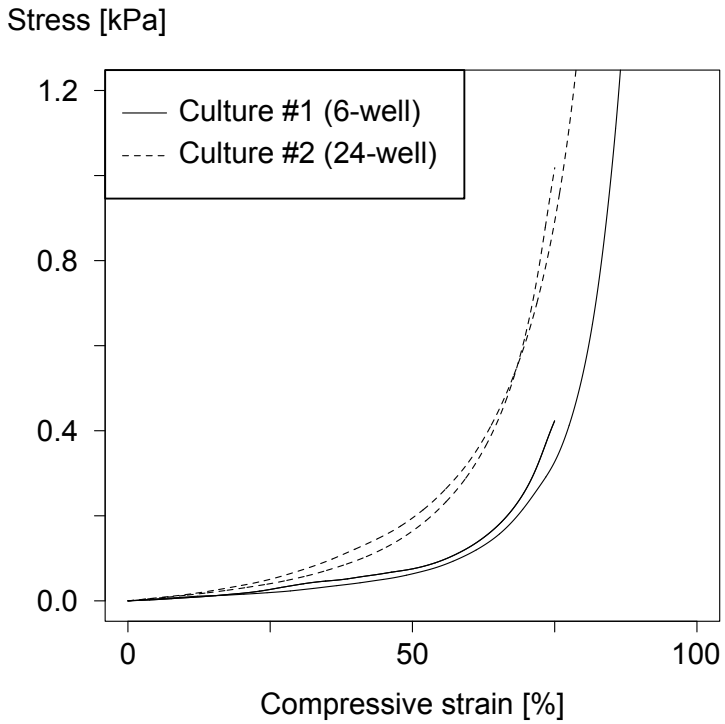

Fig. S1-7. Uniaxial compression: Stress-Strain diagram. Four measurements were done: to 75% compression, and then to 100% compression on a total of two samples (one reconstituted from each culture condition).

Fig. S1-7 shows the stress-strain diagrams for the four compression experiments: to 75%, and then again from original sample height to 100% compression, for the two samples prepared (one from the 6-well culture, one from the 24-well culture).

All four compression experiments show a qualitatively similar behavior in compression: with increasing strain, the elastic recoil force raises more and more steeply. Quantitatively, the compression curves show high repeatability within each sample, as the compression to 100% recapitulates closely the previous compression to 75% on the common part from 0 to 75% compressive strain. This shows the very good elastic recovery typical for cryogels. There is a more important difference between the first and second sample, the second appearing stiffer. This is most likely rooted in a difference in particle concentration achieved during loading. This phase is indeed manually controlled by the effective dehydration pressure applied, which is only controlled to within visual observation, avoiding high capillary pressure as evident by the formation of strongly curved menisci in the clefts between cleaning cloth and Perspex sheet.

At least during the initial stages of uniaxial compression, we expect mostly pore fluid to be expelled from the highly porous biomaterial. This estimate the effective polymer concentration as a function of the strain as defined by eq. S1-2:

$$c = \frac{c_0}{1-\varepsilon} \qquad \text{eq. S1-3}$$

where c_0 is the polymer concentration in a reference state. We carry out the loading procedure such as to minimize capillary aspiration pressure by careful observation of the menisci. Hence, we expect a material density close to one observed during spontaneous sedimentation, which by dry mass determination is about c_0=7mg/mL crosslinked material in cell culture medium.

Noting that the concentration of polymer changes due to progressive compression allows to estimate the variation of the Young modulus at each polymer concentration from the stress-strain curves shown in Fig. S1-7. The Young modulus is defined as the change of stress per unit of change of strain:

$$E = \frac{d\sigma}{d\epsilon} \qquad \text{eq. S1-4}$$

where $\frac{d\sigma}{d\epsilon}$ is the slope of the stress-strain curves as shown in Fig. S1-7.

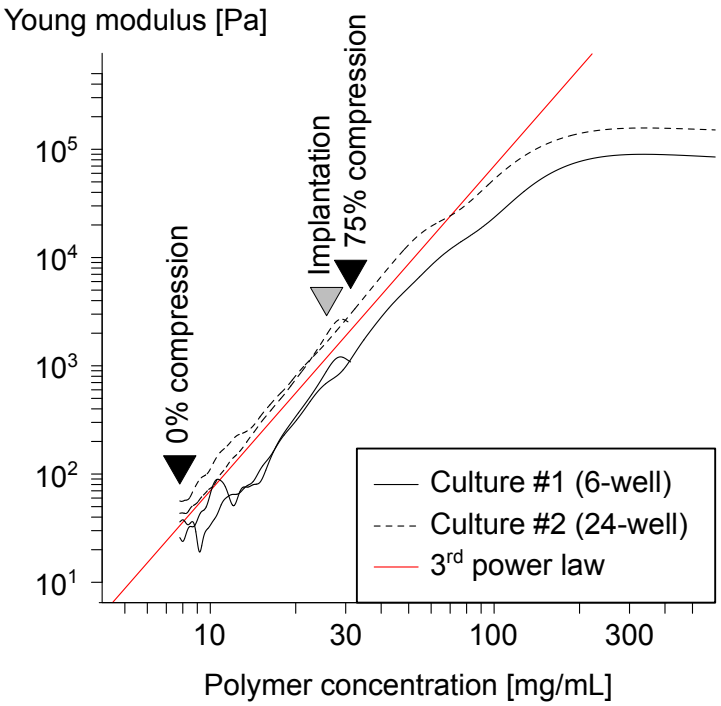

Fig. S1-8. Young modulus as a function of polymer concentration. Eq. S1-4 is used for the estimation of the Young modulus from the data shown in Fig. S1-7; eq. S1-3 is used to estimate the polymer concentration from the strain shown in Fig. S1-7. The triangles labelled "0% compression" and "75% compression" outline the estimated polymer concentrations at which the viability quantifications for the 0% compression and 75% compression conditions were done; the grey triangle labeled "Implantation" indicates the estimated concentration of the biomaterial for the in-vivo implantation experiments (26mg/mL, see supplementary 3).

Fig. S1-8 outlines the Young moduli as estimated from eq. S1-4 plotted against the polymer concentration as estimated by eq. S1-3. Over a wide range, a 3rd power law is observed for both samples, in agreement with a similar law reported for bulk cryogel scaffolds.[2] The polymer concentrations shown on the x-axis of Fig. S1-8 should be

considered indicative: It is quite difficult to determine the exact polymer concentration reached when loading the polymer chamber. For the small volumes used for the compression measurement, the mere act of transferring to a scale causes pore fluid to be lost. We performed two experiments to define the upper and lower bounds of the polymer concentration after loading.

To estimate the lower bound, we performed sedimentation over 24h of a dilute suspension of CCMs in a large excess of cell culture medium. From the volume of the sediment and the determination of the dry mass present (by the CCMs with excess deionized water followed by oven drying), we could estimate the free sedimentation concentration to be 6.0+/-0.6mg/mL.

To estimate an upper bound, we replicated the loading of the compression chamber with larger volumes (ca. 1mL) and a 40 micrometer cell strainer together with a larger mock compression chamber to be able to transfer with less change of fluid volume. The cell strainer makes the transfer to a scale for hydrated weight determination easier, although some loss of fluid is still expected. In this way, we could estimate an upper bound to the chamber loading concentration to be 9.6 +/-2.0mg/mL.

The actual polymer concentration is expected to between the upper and lower bounds thus determined. As a reasonable estimate, we indicate the range midpoint and the upper and lower bounds as extremes, and therefore assume the loading concentration to be 8+/-2mg/mL.

Using this estimation of 8+/-2mg/mL for the loading polymer concentration, Fig. S1-8 indicates that the implants have Young moduli in the lower kPa range (1.2+/-0. 6kPa). Also, there is well-respected 3rd power law for the dependency of Young modulus on the polymer concentration throughout a large part of the polymer concentration range throughout the compression experiments. We previously found a similar 3rd for bulk cryogel scaffolds.[2] The 3rd power reflects the mechanics of a porous elastic structure[2] where increasing compression leads to loss of pore fluid without major change of the mechanical structure. Important deviation from the 3rd power law only sets in above about 100-200mg/mL; this concentration corresponds about to previously reported polymer concentration within the cryogel walls of bulk scaffolds[2], and thus departure from the 3rd power-law can be taken as a sign of complete pore closure. This is in line with the viability data: at 75% compression (corresponding to about 40mg/mL polymer concentration), most of the pores conserve some pore fluid, so that the cells remain protected[2]. Pore closure and ensuing massive cell death only sets in at higher polymer concentrations still.

For *in-vivo* injections, we concentrate the CCM suspensions by applying 2 cm of water column. During this procedure, we reach a polymer concentration of about 26mg/mL (Supplementary 3). This is well within the domain of the 3rd power in Fig. S1-8, and also below the 32mg/mL associated with 75% compression where much of the viability was still conserved.

Overall, we can conclude the cells should remain protected from excessive compression in the biomaterial formulation used for *in-vivo* injections because most of the pores remain at least partially open, so that little mechanical force is transmitted to the cells.

During actual injection procedure, one needs to exclude additional sources of cell damage. For instance, larger particles may undergo higher compression, and there could be damageable friction with the catheter walls. Hence, in Fig. 4E in the main text, we directly assessed the influence of the actual injection procedure on the cells.

Bibliography

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Supplementary 2: Video demonstration of drying the scaffold particles and preparation for *in vivo* murine injection

(see attached movie)

Supplementary 3: Design and use of drying device for CCM concentration prior to injection

Introduction and aims

We perform *in-vitro* co-culture of OP9 with KLS (murine bone marrow cells, ckit$^+$, Lin$^-$, Sca-1$^+$) cells, on specific microcarriers referred to as CCMs (collagen-coated carboxymethylcellulose microscaffolds). These *in-vitro* cultures are performed in dilute suspensions. For implantation, the CCMs need to be concentrated to enable interlocking and thus the formation a tissue-like, yet injectable suspension[3]. Hence, there is a need for a partial, controlled dehydration process. This process needs to satisfy the following criteria:

1) Cell viability and co-culture ratio should be maintained
2) The concentration of CCMs in the in-vitro culture is typically only approximately known (incomplete transfer, imprecision in preparation), yet a paste-like material of repeatable CCM polymer concentration should be obtained
3) The concentrated CCMs need to be obtained in a recipient that allows for lossless transfer to subcutaneous injection

This supplementary section describes the dehydration process used to achieved the criteria 1-3 listed above. It makes use a specific dehydration device, which has been designed to dehydrate a solution of CCM at an optimal polymer concentration using hydrostatic pressure. It allows an efficient removal of the dead volume while guaranteeing a safe (criterion 1), reproducible (criterion 2) and easy transition (criterion 3) from the *in vitro* cell culture to the *in vivo* injection. The whole process is harmless for the cells to be transplanted. Dehydration is complete in few minutes and CCM are then ready to be injected in vivo. The whole system is autoclavable (all pieces are made from polypropylene plastic) and can be treated for endotoxin removal process (with concentrated sodium hydroxide) in order to prevent excessive inflammatory response from the host.

Operating principle

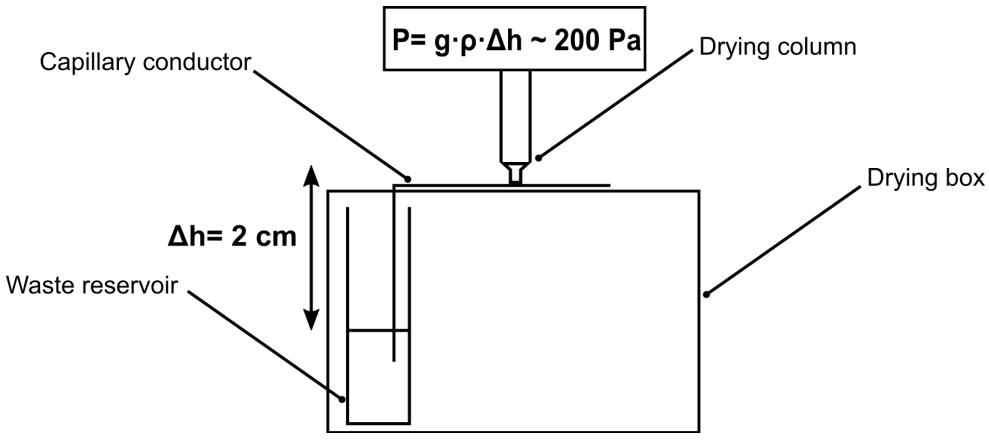

Figure S3-1: Schematic representation of the hydrostatic pressure applied by the device. A capillary conductor drives the pressure applied by the level in the waste reservoir of the drying box to the sample in the drying column. The pressure can easily be adapted by changing the level of liquid in the waste reservoir.

Figure S3-1 shows the operating principle of the drying device. The sample is applied in a drying column. Aspiration pressure is generated by a lower level of fluid in the waste reservoir of the drying box, and is transmitted to the sample via a capillary conductor (cloth). The magnitude of the aspiration pressure is set by the level of fluid in the waste reservoir. The fluid movement is determined by the relative magnitude of the elastic expansion force of the CCM sample in the drying column as compared to the imposed aspiration pressure. Therefore, after equilibration, the aspiration pressure determines the fluid content of the CCM sample regardless of the initial fluid content.

Process flow

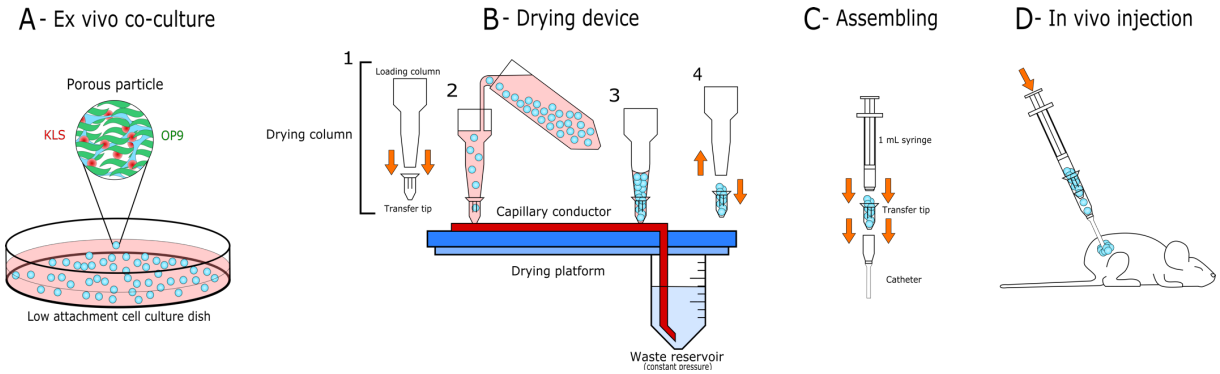

Figure S3-2: Overview of the transition from in vitro culture to in vivo injection. A) In vitro co-culture of OP9 (stromal cells) and HSPC (KLS) inside our collagen-coated CCM. The biomaterial is used as a microcarrier in a diluted form. B and C) Concentration the diluted CCM by the drying device. The protocol is detailed below. D) Subcutaneous injection of the concentrated CCM in mice.

Fig. S3-2 provides an overview over the process flow used to obtain an injectable from CCM microcarrier cultures. The starting material is a dilute suspension of CCMs colonized with the cells of interest (typically, a co-culture of HSPC and OP-9 feeder cells, Fig. S3-2A). This dilute suspension is transferred to the dehydration column, which consists itself of two parts, namely the loading column and the transfer tip (Fig. S3-2B). Excess fluid is drained into the waste reservoir by the dehydration box, until equilibrium with the aspiration pressure set by fluid level in the waste repertoire is reached (Fig. S3-2B). In this compacted stage, the CCMs form a paste-like material, contained in the transfer tip. This material holds in place, such that the loading column can be removed. The transfer tip is then attached to a syringe and equipped with an injection catheter (Fig. S3-2C). The CCM-based injectable can now be transferred *in vivo* (Fi. S3-2D).

Description and usage of the drying device

This section provides further description and a detailed usage protocol for the drying device.
The drying is composed of 2 separate parts: i) a drying column and ii) a drying box (Fig. S3-1). The drying column handles the CCMs, while the drying box is designed to

apply a precisely known aspiration pressure and to remove excess pore fluid until equilibrium with the aspiration pressure is reached.

i) Drying column

They drying columns are themselves composed of 2 parts: the loading column and the transfer tip (as shown in Fig. S3-2). The loading column is adapted to receive an important amount of volume, typically the content of a 6-well plate well. The transfer tip is plugged at the bottom part of the loading column. It acts as an adaptor for both the column and the needle. Once, the liquid has been removed and the CCM reached the right polymer concentration (26 mg/ml), the particles will remain in the transfer tip. Thus, it can be unplugged from the columns and directly re-plug on a catheter or a needle.

ii) Drying box

The box is composed of a drying platform and a waste reservoir. The capillary conductor (contacting cloth) ensures the connection between these two compartments. The waste reservoir is a Falcon tube into which a whole at a given height has been drilled to ensure a constant level of saline buffer whatever the exact amount of excess medium in the CCM solution. The excess of liquid will indeed leak out of the tube by the hole performed at a specific height defined by the experimental needs. Indeed, polymer concentration may vary from one application to another. We use here a drying box with a preset height of hole at 2cm equivalent to about 200 Pa aspiration pressure), which we find to produce a CCM concentrate suitable for a subcutaneous injection while preserving cell viability.

Usage protocol:

We use the following protocol to concentrate the CCMs into an injectable paste under sterile conditions:

1) Under sterile conditions, humidify the capillary conductor with saline buffer. Add saline buffer in the waste reservoir in order to reach the hole level.
2) Plug the transfer tip to the loading column. Then, place the drying column in the holder. Make sure that the column is in contact with the capillary conductor.
3) Using a serological pipette, transfer the content of the well in the loading column and avoid formation of air bubble in the column. Otherwise, tap gently on the column in order to remove them.
4) Dehydration should take few minutes. In the meantime, prepare the injection syringe (typically 1 ml syringe) by aspirating sequentially 150 ul of non-seeded CCMs at 26 mg/ml and 150 ul of air.
5) Once the CCM reached the desired concentration, using sterile forceps, unplug the transfer tip from the loading column. Specific care should be taken to avoid CCM fall off from the tip.
6) Plug the transfer tip containing the CCMs to the catheter or the needle. Plug the injection syringe.
7) Insert the catheter into the insertion hole (the injection site should be cleaned and insertion hole should be made before the dehydration).

8) Inject the content of the syringe.
9) Remove the catheter gently.

Fabrication details

This section provides technical details and protocols on the custom fabrication of the loading columns and the drying box.

Loading columns

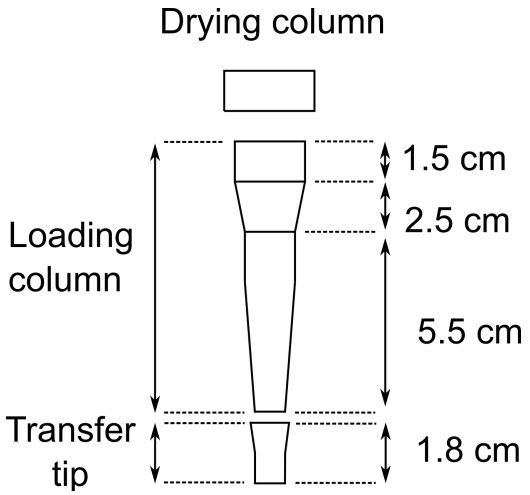

Figure S3-3: Technical details of the drying column. The drying column consists of a loading column and a transfer tip. The transfer tip is reversible attached to the loading column by pressing. Optionally, the loading column can be closed by a cap.

Figure S3-3 shows the technical dimensions of a drying column, which consists of two mandatory pieces: the loading column and the transfer tip. Optionally, it can be closed with a cap on its top.
In the following, a laboratory-scale fabrication method for the loading column and transfer tip are given.

Loading columns:

Materials

- Falcon 15 ml (bottom and top part) (PP)
- 1000 ul tip from Axygen (7.9cm) (PP)

Fabrication method

1) Cut the falcon 15 ml in 2 parts: bottom part at the 3 ml line and top part at the 12 ml line. Discard the middle part.
2) Assemble the 2 parts by melting a rim of plastic with a hot air gun, followed by rapidly pressing the two parts together.
3) Cut the very bottom part (conic part) of the falcon tube. The diameter of the hole should be the same than the top part of the 1000 ml tip
4) Assemble the tip and the falcon tube, again by melting a rim of plastic on both the tip and the shortened Falcon tube with a hot air gun followed by pressing.

5) Cut the bottom part of the tip (remove between 1.2-1.5 cm). Outer diameter 0.4 cm. Average total height size of the loading column 11.5 cm

Transfer tip:

Materials

- 20 ul from MultiGuard, Sorenson, Bioscience Inc. (PP)

Fabrication method

1) Cut the top part at 1.2 cm from the top (largest part)
2) Then cut the bottom part at 2.3 cm from the bottom (the narrowest part), below the filter.

Drying box:

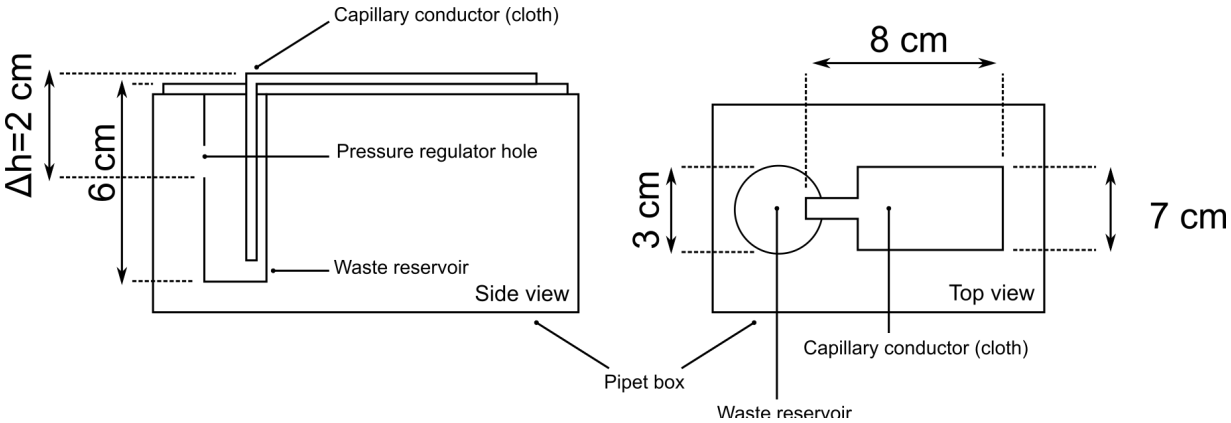

Figure S3-4: Technical layout of the drying box. The drying box a customized pipet tip box: a waste reservoir with a lateral hole for pressure regulation is inserted into tip shelf, and a capillary conductor in the form of specifically cut wiping cloth is laid out onto the plateau. An extended part of the capillary conductor extends into the reservoir to transmit the aspiration pressure set by the position of the pressure regulator hole. The pipet box also serves as a containment for the excess liquid leaving the waste reservoir by the pressure regulator hole.

Materials

- 1000 ul pipet box, Axygen scientific (PP). The tips are not needed.
- Table cleaning cloth, local supermarket (Coop, 82%viscose, 18% polypropylene, autoclavable)
- 50 mL falcon tube, Corning (PP)

Fabrication method

1) Make a hole in the tip shelf of the size of a 50 ml falcon tube
2) Insert the falcon tube and melt it with a soldering iron in order to attach it to the box
3) Make a hole in the falcon tube at the desired height (here 2 cm) from the top part. This will define the aspiration pressure
4) Contacting cloth: cut in a table cleaning cloth a rectangle of 8x7 and with a tail of at least 6 cm at one side.

Aspiration pressure and polymer concentration

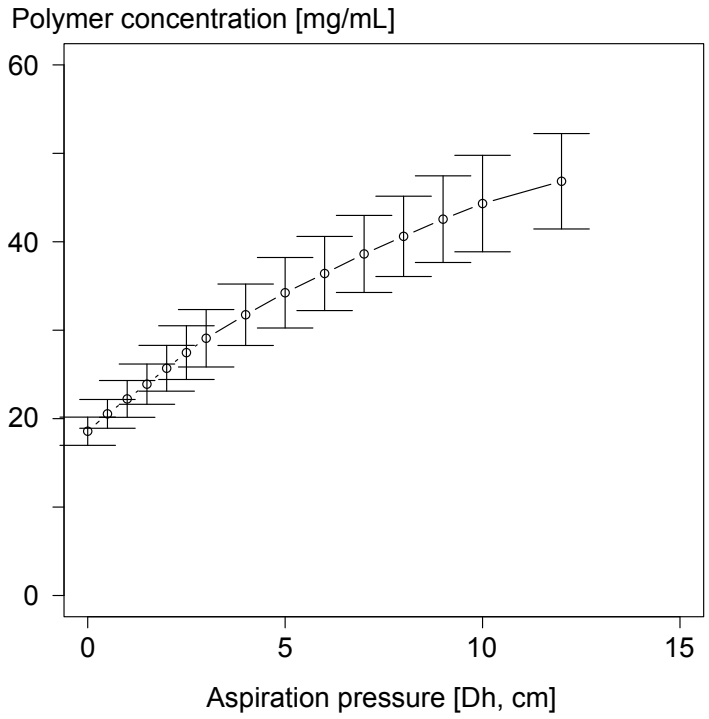

Figure S3-5: Relation between aspiration pressure and the dry weight polymer content of the CCM biomaterial (uncoated precursor). The aspiration pressure is expressed by the height difference between the pressure regulator hole and the plateau of the drying box (Δh in Fig. S3-4).

The use of the drying device hinges on the equilibrium relation between polymer concentration and aspiration pressure. To establish this relation for the biomaterial constituted by the dense suspension of CCM, we measured the dry polymer content at different preset aspiration pressures. We did so for uncoated, cell-free CCM precursor material.
For this measurement, we replaced the transfer tip by a cell strainer. In this way, we could determine the weight of material equilibrated with a predefined series of aspiration pressures, before extensive deionized water washing to remove free salts, and microwave or oven drying to constant weight for dry weight determination.
Figure S3-5 shows the relationship between the dry weight polymer concentration of the biomaterial and the aspiration pressure as set by the height difference Δh between the shelf of the pipet box and the lower edge of the pressure regulator hole. At a preset Δh of 2cm, we obtain a polymer concentration of 26 +/- 3mg/mL. This is about the double of the total polymerizable mass content of the premix used to make the bulk scaffolds before fragmentation, indicating that there is substantial compaction and probably compression of the CCMs. However, this is still substantially below the about 40mg/mL of polymer concentration where an onset of decrease in viability was seen (Supplementary 1). In any case, using the partial dehydration procedure as described in this supplementary, we did not observe a decrease in cell viability, nor a change in compositions of the co-cultures (Fig. 4E in the main text).

Reference

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3 Beduer, A., Verheyen, C. A., Bonini, F., Burch, P. & Braschler, T. *submitted* (2019).