Supplementary Information

The Effect of Dexamethasone on Room Temperature 3D printing, Rheology, and Degradation of a Low Modulus Polyester for Soft Tissue Engineering

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S.1. Construction of thermodynamic phase diagram:

According to the F-H solution theory, the dependence of χ on temperature can be predicted by mixing the drug and the polymer in different compositions and recording the endpoint melting temperature of the drug at near equilibrium conditions. Based on the obtained drug melting temperatures, χ can be predicted based on the relationship:

$$\frac{1}{T_m} - \frac{1}{T_m^o} = -\frac{R}{\Delta H}\left[\ln \Phi_{drug} + \left(1 - \frac{1}{m}\right)\Phi_{poly} + \chi_{drug-poly}\,\Phi_{poly}^{\,2}\right] \quad \text{------- (S.1)}$$

where, T_m and T_m^0 are the melting temperature of the drug in the drug-polymer mixture and the pure drug, respectively, and ΔH is the heat of fusion of the drug.

Since we 3D print the drug-polymer mixtures at ambient room temperature conditions, the χ values obtained at the high drug melting temperatures (230-270^{0}C) need to be extrapolated to lower temperatures. Although χ is a function of both temperature and composition, a simplified first order relationship between χ and temperature was shown to be sufficient to estimate χ for many polymer–polymer systems exhibiting an upper critical solution temperature (UCST),[1] expressed as:

$$\chi = A + \frac{B}{T} \quad \text{------- (S.2)}$$

This first order relationship between χ and 1/T has been used to extrapolate the value of χ outside the experimental temperature range.[2–5] The value of the entropic contribution A, and the temperature dependent enthalpic contribution B, can be determined by calculating the intercept and slope, respectively, of the plot of χ Vs

1/T as shown in equation S.2. The ΔG_{mix} as a function of temperature and composition can then be calculated by combining equation S.2 with equation S.3.

The change in Gibbs free energy of mixing of a solute (drug) in a polymer may be expressed as a function of χ as:

$$\frac{\Delta G_{mix}}{RT} = \phi_{drug} \ln \phi_{drug} + \frac{\phi_{poly}}{m_{poly}} \ln \phi_{poly} + \phi_{drug}\phi_{poly}\chi_{drug-poly} \quad \text{------ (S.3)}$$

where Φ_{drug} and Φ_{poly} are the volume fraction of a drug and polymer, respectively, R is the universal gas constant, T is the temperature, and m is the ratio of the volume of a polymer chain to the volume of a drug molecule.

The drug solubility in the polymer is given by the binodal curve, which can be constructed by equating the chemical potentials ($\partial \Delta G_{mix}/\partial \phi_i$) in the drug-rich and polymer-rich phases for both i species, under the constraint $\sum_i \phi_i = 1$ in each phase. The resulting system of equations can be solved using a standard non-linear least squares minimization routine at each temperature. Moreover, the drug-polymer miscibility boundary (spinodal curve) can be calculated by setting $\partial^2 \Delta G_{mix}/\partial \phi_i^2$ equal to zero (again under the constraint $\sum_i \phi_i = 1$) and solving for the roots of the resulting equation. A detailed discussion of the theoretical considerations and the calculations can be found elsewhere.[1,6]

S.2. Discussion of drug – polymer phase diagram:

Melting point depression of drugs is generally observed after solid state processing, like ball milling, where the crystal size and the amorphous-to-crystalline drug ratio gets altered.[3] Thus, the decrease in size and crystallinity lowers the chemical potential and, therefore, depresses the drug melting temperature.[3,1] Likewise, the partial dissolution of a crystalline drug in a polymer matrix reduces the drug's chemical potential resulting in a decreased melting temperature.[1] The endpoint melting temperature is used to determine the complete dissolution of the drug for each composition.[1,7] This melting point depression is used to estimate the dissolution limit or solubility of a crystalline drug in the polymer.

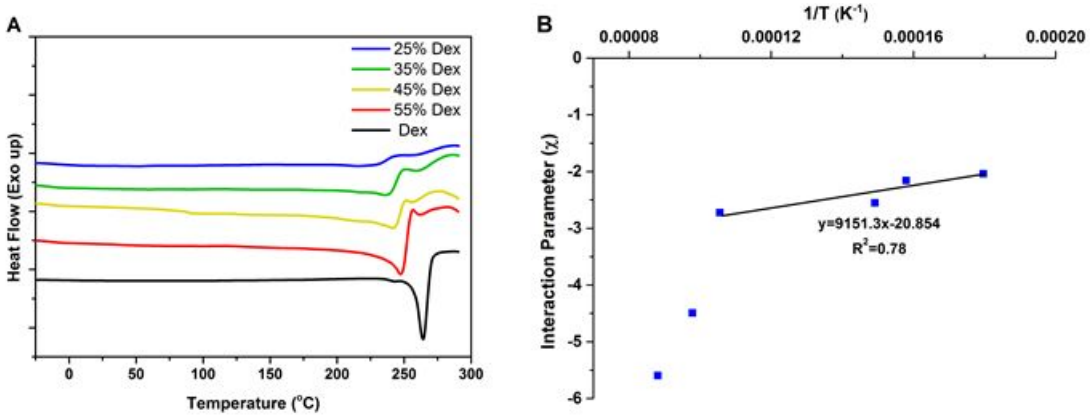

Figure S.1.: A) Melting point depression of Dex physically mixed in the SC5050 polyester; B) Interaction parameter as a function of temperature. The linear line represents the fitting of experimental data to find A and B coefficients in Eq. 2.

Figure S.1.A shows the DSC thermograms of various compositions of physically mixed Dex in SC5050 polyester. Clearly, there is significant evidence of melting point depression which suggests favorable drug/polymer interaction at the drug melting temperature.[8] The end-set melting point of Dex decreases with an increasing fraction of the SC5050 polyesters with no clear melting event observed for compositions below 35% w/w drug-polymer mixtures. The depression in the end-set melting temperature was combined with equation 1,

$$\frac{1}{T_m} - \frac{1}{T_m^o} = -\frac{R}{\Delta H}\left[\ln \Phi_{drug} + \left(1 - \frac{1}{m}\right)\Phi_{poly} + \chi_{drug-poly}\,\Phi_{poly}^2\right] \quad \text{------- (S.1)}$$

to calculate χ at the melting temperature.

We assume that for the Dex-SC5050 binary system the F-H interaction parameter may be described to have a first order relationship with inverse temperature with relatively negligible dependence on composition, shown as,

$$\chi = A + \frac{B}{T} \quad \text{------- (S.2)}$$

Similar assumptions have been made for other drug/polymer systems. We note that the relationship described by equation 2 has not been validated at low temperatures. The plot of χ versus 1/T is shown in Figure S.1.B. While a non-linear relationship was observed over the whole range of concentrations studied, a linear relationship was observed for the lower composition range (35%-55%). Non-linearity between F-H interaction parameter and 1/T at high concentrations has been previously reported for other similar drug/polymer systems[3,1] It is suggested that the observed non-linearity at high drug compositions may be due to the dependence of the interaction parameter on higher order concentration terms in specific drug/polymer systems.[3,1] Moreover, since the non-linearity is only observed at small values of 1/T and thus high temperatures, it is assumed the linear relationship will hold at low temperatures of interest in this case.[3] Given this assumption, the values of F-H constants A and B were calculated by a linear regression fitting of equation S.2 to the data in Figure S.1.A. Equation S.2 gives the relationship of χ with temperature with the obtained values for the constants A (-20.854) and B (9151.3). A negative value of χ indicates perfect mixing with complete solubility of the drug in polymer, whereas a high positive value would indicate drug-polymer immiscibility. In the present case, a high positive value of χ (~9.8 at 25°C) suggests Dex is immiscible in the SC5050 polyester at ambient temperatures used for 3D printing.

By substituting the values of χ given by equation S.2 into equation S.3,

$$\frac{\Delta G_{mix}}{RT} = \phi_{drug} \ln \phi_{drug} + \frac{\phi_{poly}}{m_{poly}} \ln \phi_{poly} + \phi_{drug}\phi_{poly}\chi_{drug-poly} \quad \text{------ (S.3)}$$

the change in Gibbs free energy as a function of temperature and composition is calculated and shown in Figure S.2.

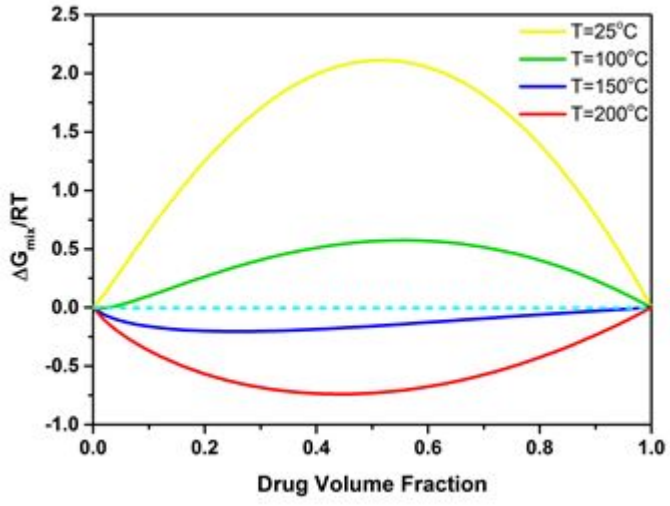

Figure S.2: Plot of Gibbs free energy as a function of the drug volume fraction and temperature for the Dex-SC5050 system.

S.2. Deconvolution of UV-Vis spectra for drug release:

The broad absorption spectra from the collected solutions was deconvoluted by first removing the background, which was determined by a linear fit to the region of spectra (> 400 nm) where no signal was present. Next, we assumed that each of the i individual peaks could be represented as a Gaussian distribution with a maximum absorbance α_i^0 at λ_i^{max} and standard deviation σ_i, such that the complete spectra were described by:

$$\alpha(\lambda) = \sum_i^n \alpha_i^0 \exp\left[-\frac{(\lambda - \lambda_i^{max})^2}{\sigma_i^2}\right] \quad \text{----- (S.4)}$$

where $n = 5$. Using the λ_i^{max} specified above, the remaining unknown α_i^0 and σ_i where then determined for each spectrum using a standard non-linear least square fitting routine. We note that the spectra were well fit by Eq. S.4, with chi-squared values typically around 10^{-3} and quantitatively consistent σ_i for each compound in all fit results.

The best fit α_i^0 were then converted to concentration using the results of standard curves obtained using solutions of Dex, soybean monomer (S), coumarin monomer (C), and adipic acid.

S.3. Estimating SC5050 degradation by UV-Vis Spectroscopy:

The standard curves of monomers were used to estimate the amount of each monomer of the SC5050 polyester released in the PBS media. The coumarin (C) monomer had a distinct absorption peak at 249nm (λ^{max}) whereas the λ^{max} for the soybean monomer (S) and the adipic acid are both 203 nm. However, directly adding the amount of each monomer released in PBS resulted in erroneously high amounts of released SC5050 polyester. This is because our method overestimates the amount of adipic acid present in the collected media. Moreover, the degradation products of the SC5050 polyester have another peak at 275nm which corresponds to the crosslinked coumarin dimers. Since a standard curve could not be made for coumarin dimer, the amount of coumarin dimer in degraded polyester could not be determined. Because the estimated amounts of soybean monomer appeared to be most reasonable, the amount of SC5050 polyester was estimated based on the amount of soybean (S) oil released. The amount of the other two monomers (coumarin and adipic acid) was then estimated based on the amount of soybean (S) monomer and the feed stoichiometry of the SC5050 polyester. We note that based on the nature of the monomer and it's solubility in the release media, the amount of one monomer released in solution may not be directly equivalent to the released amount of other monomers. However, the total amount of polymer released calculated by this method matched well with the mass loss of the polymer scaffolds measured by weighing the scaffolds before and after the in-vitro release experiment (see Figure S.5.).

S.4. UV-Vis spectrum of collected samples:

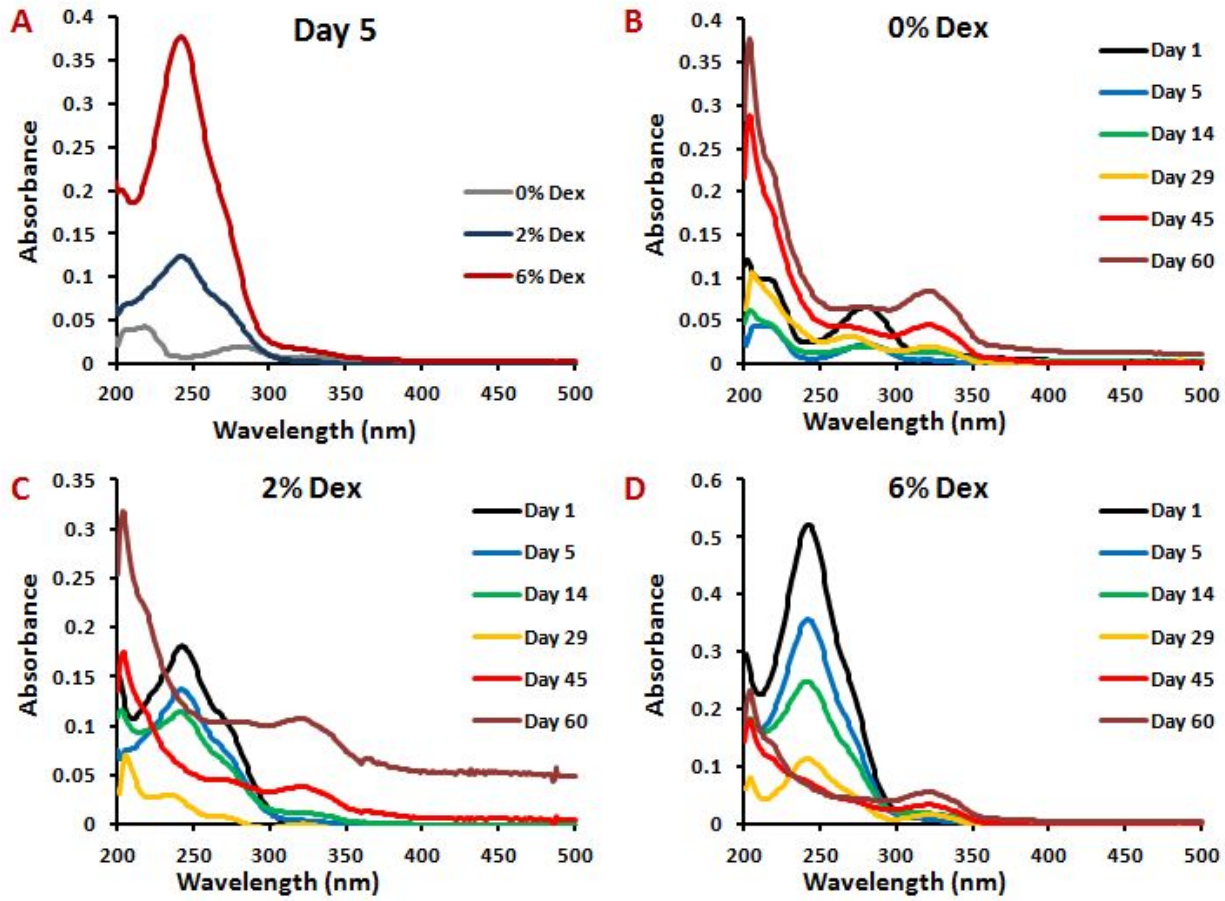

Figure S.1.: Representative UV-Vis absorption spectrum of the collected samples of - A) 0%, 2%, and 6% w/w Dex samples at day 5; B) 0% Dex, C) 2% Dex, and D) 6% Dex at various days for the drug release and polymer degradation *in-vitro* experiment.

S.5. Drug Release standard curves:

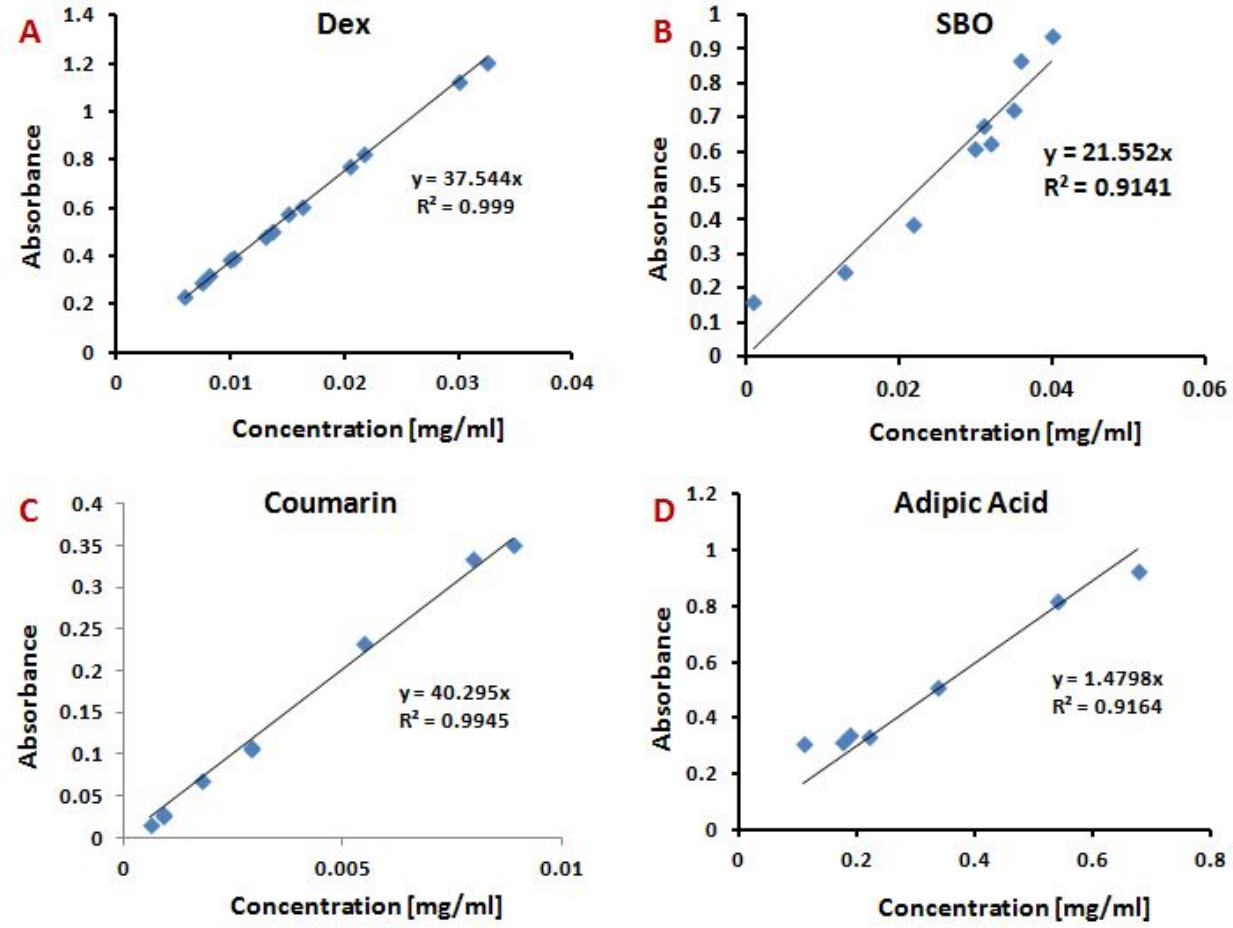

Figure S.4.: Standard calibration curves for A) dexamethasone, B) soybean monomer (SBO), C) coumarin monomer, and D) adipic acid dissolved in PBS at room temperature.

S.4. Weight loss due to in-vitro degradation of the 3D printed scaffolds:

The weight of the 3D printed scaffolds was recorded before, and after *in-vitro* degradation in PBS at 37°C for 60 days. The scaffolds were rinsed with water and lyophilized overnight to remove any residual water before weighing them after the in-vitro release experiment

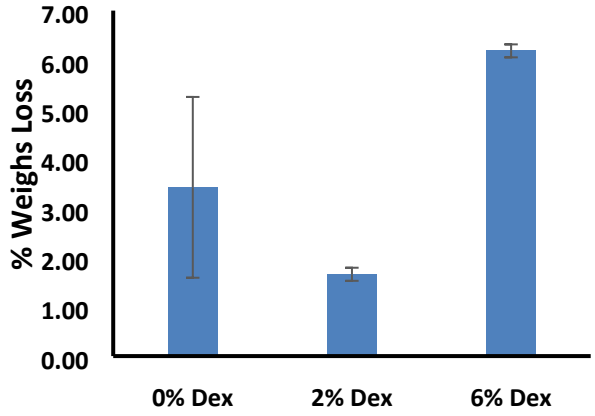

Figure S.5.: The weight loss of the 3D printed scaffolds after *in-vitro* degradation in PBS at 37°C for 60 days.

S.5. Dex release rates:

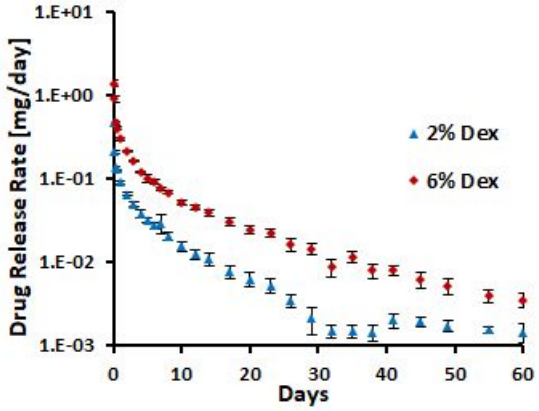

Figure S.6: Dex release rate from the 3D printed scaffolds in PBS buffer at 37C over 60 days.

S.6. SC5050 degradation rate:

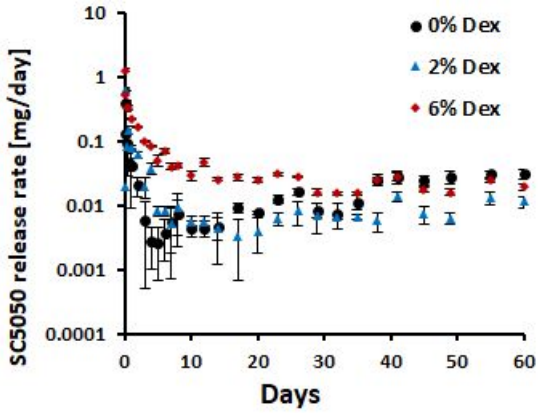

Figure S.7: SC5050 release rate from the 3D printed scaffolds in PBS buffer at 37C over 60 days.

S.7. SC5050 degradation curve fitting:

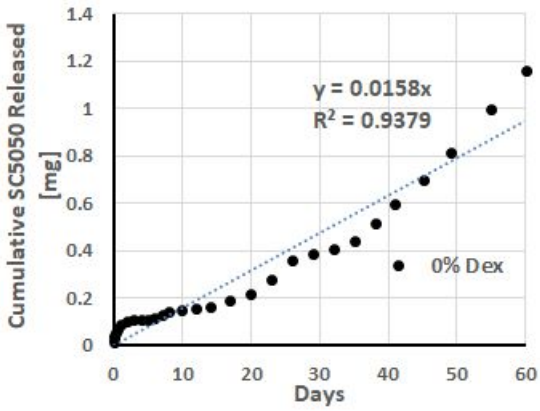

Figure S.8.: Linear regression fitting of the SC5050 polyester released in PBS during the drug release experiment.

S.8. Molecular weight determination:

Polyester molecular weights were analyzed on a Viscotek TriSEC equipped with an RI and UV detector. Separation occurred over two PSS Gram Analytical GPC Columns in series using THF as eluent at a flow rate of 0.8 mL/min. The column and detector temperatures were maintained at 50 °C. Molecular weights were obtained relative to PS standards.

S.9. NMR:

NMR spectrum of the SC5050 polyester and monomers were recorded on a Varian Mercury 300 or 500MHz spectrometer in $CDCl_3$. All the spectrums matched the spectrums shown in our previous work [7].

S.10. SEM images of the cross-section of the 3D printed scaffolds:

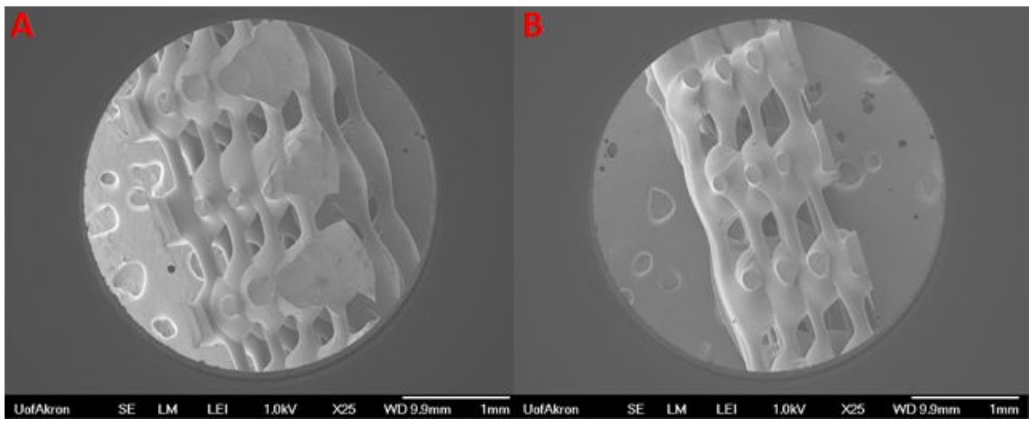

Figure S.8.: SEM images of the cross-section of the 3D printed scaffolds of A) SC5050, B) SC5050 + 6% w/w Dex.

S.11. Image analysis of collagen surface area in tissue sample:

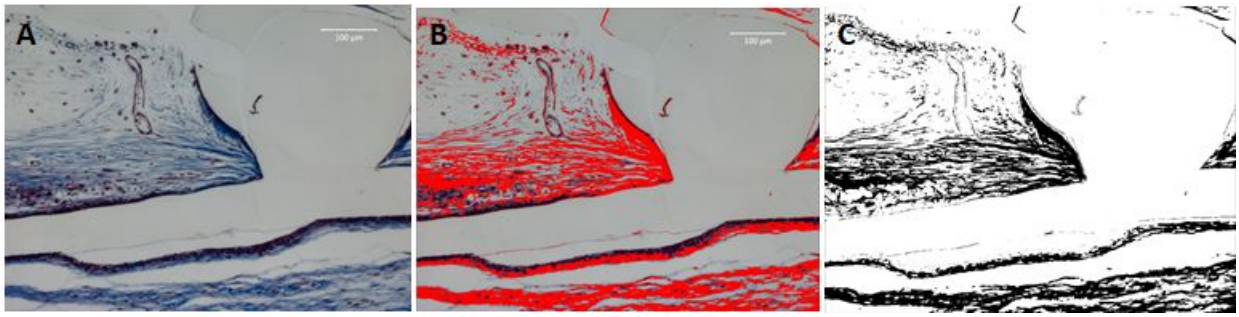

Figure S.9.: ImageJ analysis of collagen surface area in tissue sample. (A) Original picture showing collagen in blue. (B) Picture after color thresholding. (C) Picture after binary transformation: blue pixels are seen black and all others white. The number of black pixels was measured as percent of picture area.

S.12. Zoomed-in image of the tissue samples highlighting the blood vessels:

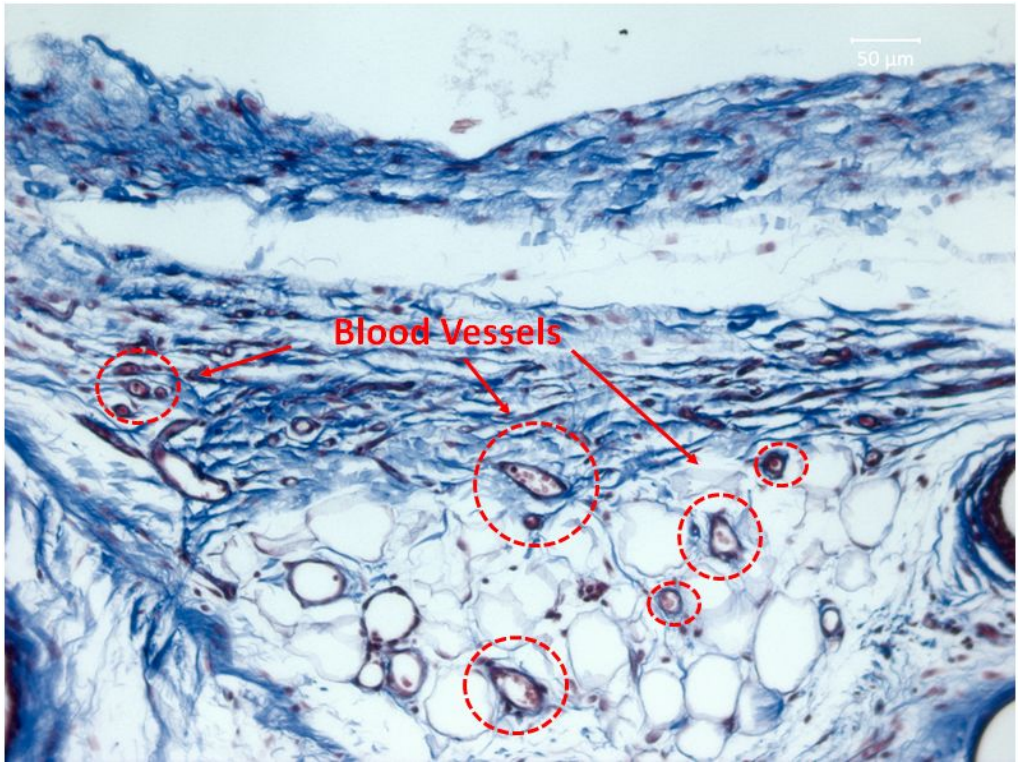

Figure S.10.: Zoomed-in image of the tissue sample highlighting the blood vessels.

S.13. Masson Trichrome stained images of the tissue samples:

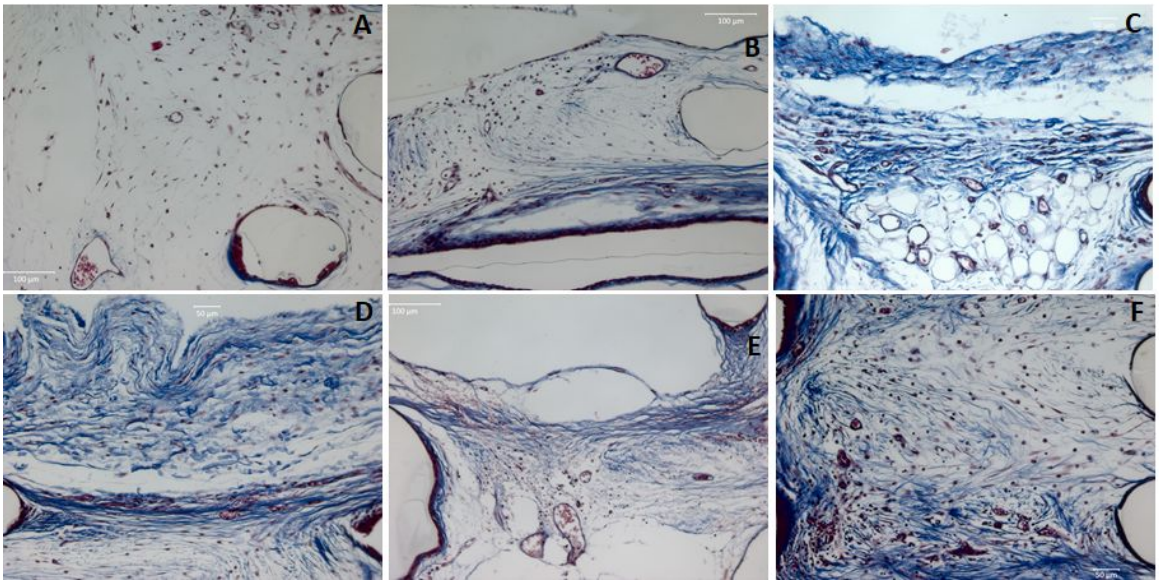

Figure S.11.: Additional images of the Masson Trichrome stained tissue samples derived after implanting the SC5050 scaffolds subcutaneously in Sprague Dawley Rats for 6 weeks.

References:

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