Supplemental Information

Use of protein-engineered fabrics to identify design rules for integrin ligand clustering in biomaterials

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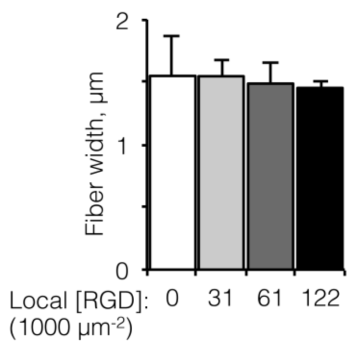
RGD-elastin in elastin blend of fibers with ligands	Local [RGD], 1000 μm^{-2}	Nearest RGD spacing, nm	Fibers with ligands in mixed fabric	Global [RGD], 1000 μm^{-2}
0%	0	n/a	0%	0
3%	8	14.4	100%	4
6%	15	11.6	100%	9
13%	31	9.1	25%	4
13%	31	9.1	50%	9
13%	31	9.1	100%	18
25%	61	7.2	25%	9
25%	61	7.2	50%	18
25%	61	7.2	100%	35
50%	122	5.7	25%	18
50%	122	5.7	50%	35
50%	122	5.7	100%	71

Supplemental Table 1. Summary of fabrication parameters and resulting local and global ligand densities.



Supplemental Figure 1: Fiber width was unchanged for fibers produced from various RGD-/RDG-elastin blends (n = 3 fabrications per condition).

Supplemental Information S1: Example global ligand density calculation

Measure bulk mass swell ratio:

$$12.0 \pm 0.3 \frac{g\ wet}{g\ dry}$$

Convert to elastin volume fraction (bulk):

$$\frac{1\ g * \frac{1\ mL\ elastin}{1.35\ g\ elastin}}{11\ g\ water * \frac{1\ mL\ water}{1\ g\ water} + 1\ g\ elastin * \frac{1\ mL\ elastin}{1.35\ g\ elastin}} = 6.3\% \frac{mL\ elastin}{mL\ bulk}$$

Measure fiber volume fraction:

$$58 \pm 4\% \frac{mL\ fibers}{mL\ bulk}$$

Calculate fiber volume swell ratio

$$\frac{6.3\% \frac{mL\ elastin}{mL\ bulk}}{58\% \frac{mL\ fibers}{mL\ bulk}} = 11\% \frac{mL\ elastin}{mL\ fiber}$$

Convert to elastin density (fiber):

$$0.11 \frac{mL\ elastin}{mL\ fiber} * 1.35 \frac{g\ elastin}{mL\ elastin} = 0.15 \frac{g\ elastin}{mL\ fiber} * 10^{12} \frac{pg}{g} * 10^{-12} \frac{mL}{\mu m^3} = 0.15 \frac{pg\ elastin}{\mu m^3\ fiber}$$

Convert to [RGD], assuming fiber is 13% RGD-containing elastin (0.52 fmol RGD/fmol elastin):

$$0.15 \frac{pg\ elastin}{\mu m^3\ fiber} * \frac{fmol\ elastin}{33.3\ pg\ elastin} * \frac{0.52\ fmol\ RGD}{fmol\ elastin} * \frac{mol}{10^{15}\ fmol} * \frac{6.02 * 10^{26}}{mol} = \frac{2.9 * 10^6}{\mu m^3\ fiber}$$

Calculate accessible [RGD] (surface concentration):

$$\frac{2.9 * 10^6}{\mu m^3\ fiber} * 0.023\ \mu m = \frac{65 * 10^3}{\mu m^2\ fiber}$$

Calculate global [RGD]:

$$\frac{65 * 10^3}{\mu m^2\ fiber} * 58\% \frac{\mu m^2\ fiber}{\mu m^2\ matrix} * 50\%\ (undersides\ of\ fibers\ are\ inaccessible) = \frac{18 * 10^3}{\mu m^2\ matrix}$$

Supplemental Information S2: MATLAB script used to generate images of segmented vinculin puncta

```
%% Vinculin Puncta Segmentation
% This script segments confocal max projections of HUVECs stained for
% vinculin and outputs a binary image of individual puncta.

clear
clc
close all

% Read in confocal max projection image and adjust contrast
filename = 'vinctest.tif';
img = imread(filename);
imshow(img);
img = imadjust(img,[0.4 1],[]);
bw = im2bw(img);

% Segment vinculin puncta from peripheral structures (fibers, etc.). Note:
% strel structuring element radii are set to 0 here but should be optimized
% for the input image.
seD = strel('diamond',0);
bw = imerode(bw,seD);
bw = imerode(bw,seD);
se90 = strel('disk',0);
se0 = strel('disk',0);
bw = imdilate(bw,[se90 se0]);
bw = imfill(bw,'holes');

% Binarize image and detect object areas, perimeter, and centroid locations
IL = bwlabel(bw);
stats = regionprops(bw,'Area','Perimeter','Centroid');
% Circularity = 4*pi*area/(perimeter^2)
objareas = [stats.Area];
objperim = [stats.Perimeter];
objcirc = 4*pi*objareas./(objperim.^2);

% Remove objects outside of defined circularity and area ranges
ind = find(objcirc >= 0 & objareas < 80 & objareas > 3);
Iout = ismember(IL,ind);
figure
imshow(Iout)

% Optional: Draw object outlines and overlay onto input image
perim = bwperim(Iout);
img_perim = img;
img_perim(perim) = 0;
figure
imshow(img_perim)
```