Read the following article: Martino et al. Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing, *Sci Transl Med* (2011), and answer the following questions. For some questions, you may need to look into referenced papers or outside material for additional information.

- [http://stm.sciencemag.org/content/3/100/100ra89.full](http://stm.sciencemag.org/content/3/100/100ra89.full)
- [http://stm.sciencemag.org/content/3/100/100ra89/suppl/DC1](http://stm.sciencemag.org/content/3/100/100ra89/suppl/DC1)

a) (2 points) What material system did the authors use?

Fibrin matrix. (2 points)

b) (3 points) Is this material system degradable? If so, by what process does the material degrade?

Yes. (1 point)
Degradation by cell/tissue proteases, including primarily plasmin. (2 points)

c) (9 points) The recombinant fibronectin that they developed had separate functional domains. List each of these domains and the rationale for including them.

1) Factor XIIIa substrate fibrin-binding sequence. (1 point)

Rationale: In the presence of Factor XIII, this sequence leads to the covalent attachment of the recombinant fibronectin to the fibrin matrix, and keeps the fibronectin within the matrix. (2 points)

2) 9th to 10th type III FN repeat (FN III9-10), integrin binding domain. (1 point)

Rationale: This domain is the major integrin binding domain. Integrins are cell adhesion receptors, so this domain promotes cell adhesion as well synergistic signaling with the growth factor binding domain. (2 points)

3) 12th to 14th type III FN repeat (FN III12-14), growth factor binding domain. (1 point)
Rationale: This domain binds many different growth factors, including several explored in this study. By binding growth factors, this domain acts to present growth factors locally to cells in close proximity to the integrin adhesive interactions. (2 points)

d) (4 points) In these studies, what is the cellular receptor that plays the most significant role in binding 9th to 10th type III FN repeat (FN III9-10) domain? How did they determine this?

Integrin alpha5 beta1. (2 points)

In several different experiments, the authors used a blocking antibody to alpha5 beta1 to demonstrate that it played a significant role in mediating the positive effects resulting from the presence of the recombinant fibronectin. (2 points)

(Inhibition of alphav beta3 with a blocking antibody demonstrated a modest effect in some experiments, but this effect was less significant).

e) (4 points) Briefly describe the assays that they used to evaluate the effects of their system on endothelial cell and smooth muscle cell function in vitro.

Endothelial cells: In vitro tube formation assay. Endothelial cells are encapsulated within fibrin matrices +/- various fibronectin constructs. These systems are cultured +/- VEGF-A165. Matrices were fixed and stained and tube-like morphology was evaluated by measuring tube length in fluorescent microscopy images. (2 points)

Smooth muscle cells: In vitro sprouting assay. Smooth cells are encapsulated within fibrin matrices +/- various fibronectin constructs. These systems are cultured +/- PDGF-BB. Matrices were fixed and sprouting behavior was evaluated by measuring sprout length in bright-field microscopy images. (2 points)

f) (4 points) What injury model systems did they use in these studies? Specifically list what animals, what tissues, and how they generated the injuries.

Wound healing model: db/db mouse (genetic model of diabetes with delayed wound healing); full-thickness back-skin wound; mouse backs were shaved and full-thickness punch biopsy wounds were made (4 per animal). (2 points)
Bone regeneration model: rat; calvarial defect; two 6-mm-diameter craniotomy defects were made in parietal bones of the skull (8-mm craniotomy at junction of ossa frontalia and ossa paritealia was also used and can be accepted as correct). (2 points)

g) (4 points) In one of the model systems, they investigated the presence of CD31+ cells. What two assays did they perform to evaluate the presence of these cells, and what advantage/disadvantage does each of these assays exhibit?

Flow cytometry: Advantage: quantitative measurement of the % of CD31+ within the explanted matrices; Disadvantage: No information on the location of the cells. (2 points)

Staining of tissue/matrix sections: Advantage: provides relative location of cells and structural information; Disadvantage: difficult to obtain quantitative evaluation of relative cell %’s. (2 points)

h) (3 points) What was the rationale for including the functional domains within a single recombinant protein (i.e. why not distinct molecules which would be added separately)?

Presentation of the functional domains within the single recombinant protein (and therefore, in close proximity) causes sequestration of integrins and growth factor receptors on cells. This sequestration leads to enhanced proliferation, migration, and differentiation of cells. (3 points)

i) (2 points) Why would it be important/necessary to reduce the concentration of clinically-administered growth factors?

High concentrations of growth factors have been associated with increased cancer risk, and can be a major safety concern. (2 points)