AD-A141 897 FORMATION AND REMODELING OF THE EARLY WOUND MATRIX(U) 1/1
TEXAS UNIV MEDICAL BRANCH AT GALVESTON P H WEIGEL
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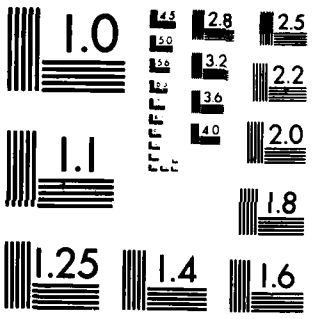

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ANNUAL PROGRESS REPORT

for the period ending 84 April 30

ONR Contract Number N00014-82-K-0279
(82 May 01 to 84 April 30)

Title: "Formation and Remodeling of the Early Wound Matrix"

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PROGRESS REPORT

a. Publications

The following publications, which have been submitted or are in preparation, have been supported by this Contract during the last 2 years.

1) Raja, R., LeBoeuf, R.D., Stone, G.W. and Weigel, P.H. (1984) Synthesis of alkylamine and ^{125}I-radiolabelled derivatives of hyaluronic acid uniquely modified at the reducing end. Analytical Biochemistry (in press)

2) Raja, R. and Weigel, P. (1983) Covalent immobilization of hyaluronic acid oligosaccharides, proteins and amino-molecules on synthetic culture surfaces. J. Cell Biol. 97, 314a.

3) LeBoeuf, R.D., Raja, R., Fuller, G. and Weigel, P. The specific interaction between hyaluronic acid and fibrinogen. (in preparation)

4) Raja, R., Grissom, M., Herzig, M. and Weigel, P. Preparation of synthetic culture surfaces: A general system for the covalent immobilization of proteins, oligosaccharides and amine-containing molecules on a non-ionic matrix. (in preparation)

A brief summary of our results and progress ~~toward the initial goals of the contract~~ is presented ~~below.~~

b. Synthesis of ^{125}I-Hyaluronic Acid Oligosaccharides

In order to detect and quantitate specific interactions of serum or cell proteins with hyaluronic acid (HA), we have synthesized a uniquely modified derivative; HA-amine. The details are presented in a manuscript which is in press and will be forwarded shortly as a Technical Report. The significance of this derivative, which has not been made before, is twofold. (i) The HA-amine derivative can be converted to a hydroxyphenyl derivative and then radiolabelled with ^{125}I at a unique position in the molecule. This HA derivative has a specific activity 10-1000 times greater than anything previously available and, (ii) the HA-amine oligosaccharides can be coupled to CNBr-activated Sepharose to make affinity chromatography media. This is the first HA-affinity support in which the oligsoaccharide is covalently attached to the matrix by a single bond at a known position (in this case, the reducing end). This method preserves the native structure of HA. We believe that the development of the HA-amine derivative will prove extremely valuable to us and other investigators in this area.

c. Development of Type I and Type II Fibrin Matrices

In our original proposal we defined Type I fibrin-based matrices as being made from pure fibrinogen and Type II matrices from cell-free plasma. Two goals of the proposal were to study the

binding of HA to these matrices and to assess their ability to stimulate the synthesis of HA by various blood cells trapped within the matrix. For these purposes we did a series of studies on the physical properties and stability of Type I and II matrices. We have defined conditions (e.g. the fibrinogen concentration range) for the preparation, washing and handling of matrices in tubes and in 35 and 60 mm culture dishes. Preliminary experiments with ^{125}I-HA and Type I and II matrices suggest that HA and fibrin interact in a specific and high affinity manner. These experiments were technically difficult to perform and evaluate although the results were consistent with our initial working hypothesis that HA and fibrin interact. Both of these molecules are very large, potentially multivalent and have the ability to form gels. Consequently, the HA-fibrin interaction is not as easy to study as, for example, the interaction between a soluble univalent ligand and a protein. We therefore decided to simplify the system and evaluate other ways to study HA-fibrinogen interactions (see below).

d. (3) Cell Interactions with Type I and II Fibrin Matrices

Conditions have been determined for culturing cells with Type II fibrin matrices formed in 35 mm culture dishes. In these experiments, human foreskin fibroblasts were used as a model cell type. Cells cultured on these matrices attached and looked healthy. Cells were also grown on tissue culture plastic and the matrix was formed on top of them or they were present during formation of the matrix and were suspended in it. In all cases, cells were viable and could be maintained in culture. Technically therefore we can do the proposed studies with blood cells to assess the effect of the extracellular matrix on HA synthesis. An assay for quantitating HA synthesis has also been adapted. These matrices can also be used to examine the ability of cells, principally fibroblasts, to migrate into and within the matrix (clot) and to screen for factors which affect this critically important process.

e. (4) Synthesis of Cell Culture Surfaces Containing Immobilized HA

Synthesis of the unique HA-amine, described above, has allowed us to make flat cell culture surfaces with this extracellular matrix molecule covalently attached. This system is based on polyacrylamide chemistry and allows one to control the chemical nature of the matrix and the concentration of the immobilized ligand. This work, supported in part by this Navy Contract, is presently being written up for publication. These culture surfaces will be used to study cellular interactions with HA and the role of HA during wound healing.

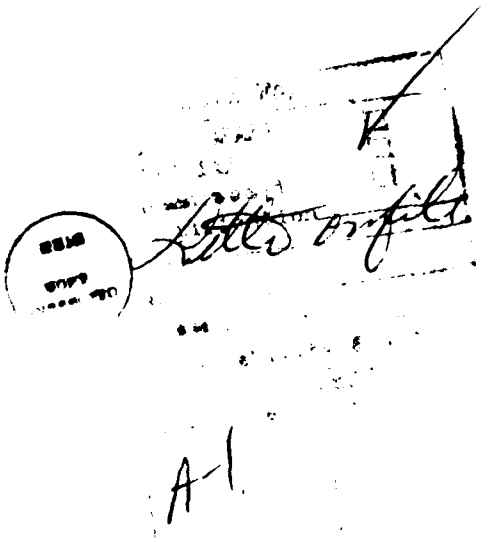
f. (5) Affinity Chromatography of Fibrinogen on HA-Sepharose

Although we observed that ^{125}I-HA bound to fibrin matrices, the presence of other proteins as well as the polymeric and insoluble state of fibrin made these experiments difficult to interpret. To overcome these difficulties, the previously described HA-amine derivative was coupled to CNBr-activated Sepharose and

used as an affinity chromatography resin to study the binding between HA and fibrinogen, the soluble monomeric form of fibrin.

Initial experiments demonstrated that when a solution of fibrinogen (2.5 mg/ml) was appllied to a 1 ml column of HA-Sepharose (1.3 mg HA/ml resin) an average of 1.35 mg of fibrinogen (SEM = 0.25 mg, n = 2) was retained by the column. The possibility that the observed binding was nonspecific was tested through two independent approaches. First, we examined whether the HA-fibrin binding was due to ionic interactions between these two macromolecules by attempting to elute bound fibrinogen with 2 M NaCl. This treatment eluted <5% of the bound fibrinogen indicating that HA-fibrinogen binding was not due to simple electrostatic interactions. Secondly, we tested whether fibrinogen would bind to the CNBr-activated Sepharose resin itself, since this support is positively charged and could interact with fibrinogen in a non-specific manner. Normally, when preparing HA-Sepharose, any unreacted sites are coupled to ethanolamine. Therefore as a control we prepared ethanolamine-Sepharose and compared the binding of fibrinogen to this resin and to HA-Sepharose. The amount of fibrinogen bound to ethanolamine-Sepharose was 17% of that bound to HA-Sepharose. Thus 83% of the fibrinogen bound to HA-Sepharose was due to specific interactions between hyaluronate and fibrinogen.

These and other experiments have demonstrated that fibrinogen and HA interact specifically, as our wound healing predict. We are presently writing up these results for publication.



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