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FORMATION AND REMODELING OF THE EARLY WOUND MATRIX(U)
TEXAS UNIV MEDICAL BRANCH AT GALVESTON DEPT OF HUMAN
BIOLOGICAL CHEMISTRY AND GENETICS P H WEIGEL 11 AUG 86
N00014-82-K-0279

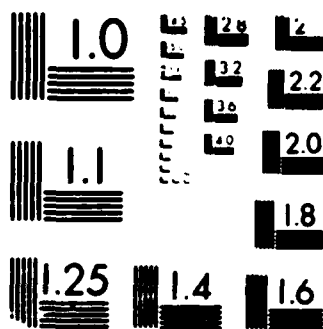
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FINAL PROGRESS REPORT
for the period ending 86 April 30

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ONR Contract Number N00014-82-K-0279
(82 May 01 to 86 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 published or are in preparation, have been supported by this Contract.

Published:

- 1) 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.
- 2) Raja, R.H., LeBoeuf, R.D., Stone, G.W. and Weigel, P.H. (1984) Synthesis of alkylamine and ¹²⁵I-radiolabelled derivatives of hyaluronic acid uniquely modified at the reducing end. *Anal. Biochem.* 139, 168-177.
- 3) LeBoeuf, R.D., Raja, R., Fuller, G.M. and Weigel, P.H. (1984) Human fibrinogen binds specifically to hyaluronic acid. *J. Cell Biol.* 99, 291a.
- 4) LeBoeuf, R.D., Gregg, R.R., Weigel, P.H. and Fuller, G.M. (1985) The effects of hyaluronic acid on the conversion of fibrinogen to fibrin and on fibrin gel structure. *J. Cell Biol.* 101, 340a
- 5) LeBoeuf, R.D., Raja, R.H., Fuller, G.M. and Weigel, P.H.. (1986) Human fibrinogen specifically binds hyaluronic acid. *J. Biol. Chem.* 261 (in press)
- 6) Weigel, P.H., Fuller, G.M. and LeBoeuf, R.D. (1986) A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. *J. Theor. Biol.* 119, 219-234.
- 7) Raja, R., Grisson, M., Herzig, M. and Weigel, P. (1986) Preparation of synthetic culture surfaces: A new reagent for the covalent immobilization of proteins and glycoproteins on a non-ionic inert matrix. *J. Biol. Chem.* 261, 8505-8513.

in preparation:

- 8) LeBoeuf, R.D., Gregg, R.R., Weigel, P.H., and Fuller, G.M. The effects of hyaluronic acid on the thrombin catalyzed conversion of fibrinogen to fibrin.
- 9) LeBoeuf, R.D., Gregg, R.R., Fuller, G.M. and Weigel, P.H. Hyaluronic acid affects the formation and structure of des AABF-fibrin gels.

- 10) LeBoeuf, R.D., Gregg, R.R., Fuller, G.M. and Weigel, P.H. Hyaluronic acid affects the rate of polymerization and structure of des AA-fibrin gels.
- 11) Frost, S., LeBoeuf, R.D., Raje, R.H. and Weigel, P. H. Preparation of hyaluronic acid oligosaccherides by controled sonication and mild acid hydrolysis.

Copies of Publications 1-7 are enclosed. When the other manuscripts crediting support by this contract are published, reprints will be forwarded to ONR.

B. Summary

The objectives of the original contract proposal were the following:

1. To characterize the specific interaction between hyaluronic acid and fibrin or various fibrin-based matrices.
2. To isolate and characterize factors from plasma, serum or cells which specifically bind to hyaluronic acid and/or alter its binding to fibrin.
3. To determine the ability of various purified blood cell types to synthesize hyaluronic acid and to determine the influence of various fibrin-based matrices on such synthesis.

The first objective was completed during the contract period and these studies resulted in the publications listed above. Due to time constraints and expansion of objective 1, only preliminary experiments were performed for the second objective and the third objectives.

Two important and novel findings were obtained as a result of this contract.

(i) Formulation of a novel model for the early stages of the wound healing process involving hyaluronic acid and fibrin.

(ii) Demonstration that fibrinogen specifically binds to hyaluronic acid.



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C. A new wound healing model

A model was presented outlining the molecular and cellular events that occur during the early stages of the wound healing process. The underlying theme is that there is a specific binding interaction between fibrin, the major clot protein, and hyaluronic acid (HA), a constituent of the wound extracellular matrix. This binding interaction, which could also be stabilized by other cross-linking components, provides the driving force to organize a three-dimensional HA matrix attached to and interdigitated with the initial fibrin matrix. The HA-fibrin matrix plays a major role in the subsequent tissue reconstruction processes. We suggest that HA and fibrin have both structural and regulatory functions at different times during the wound healing process. The concentration of HA in blood and in the initial clot is very low. This is consistent with the proposed interaction between HA and fibrin(ogen), which could interfere with either fibrinogen activation or fibrin assembly and cross-linking. We propose that an activator (e.g. derived from a plasma precursor, platelets or surrounding cells) is produced during the clotting reaction and then stimulates one or more blood cell types to synthesize and secrete HA into the fibrin matrix of the clot. We predict that HA controls the stability of the matrix by regulating the degradation of fibrin. The new HA-fibrin matrix increases or stabilizes the volume and porosity of the clot and then serves as a physical support, a scaffold through which cells trapped in the clot or cells infiltrating from the peripheral edge of the wound can migrate. The HA-fibrin matrix also actively stimulates or induces cell motility and activates and regulates many functions of blood cells, which are involved in the inflammatory response, including phagocytosis and chemotaxis. The secondary HA-fibrin matrix itself is then modified as cells continue to migrate into the wound, secreting hyaluronidase and plasminogen activator to degrade the HA and fibrin. At the same time these cells secrete collagen and glycosaminoglycans to make a more differentiated matrix. The degradation products derived from both fibrin and HA are, in turn, important regulatory molecules which control cellular functions involved in the inflammatory response and new blood vessel formation in the healing wound. The proposed model generates a number of testable experimental predictions.

D. Hyaluronic acid binding to human fibrin(ogen)

Our studies concerning hyaluronic acid (HA) interactions with fibrin(ogen) were done using the soluble monomeric form of this protein, fibrinogen. Using this approach we were able to demonstrate for the first time that HA binds specifically to fibrinogen. This interaction has been characterized and some of the molecular aspects of fibrinogen-HA binding have been determined. Since the in vivo extracellular matrix at a wound site contains fibrin we

have also assessed HA binding to fibrin. We prepared fibrin monomer-Sepharose by treating fibrinogen-Sepharose with thrombin and we then determined whether ^{125}I -HA would bind to this affinity matrix. ^{125}I -HA did bind to fibrin monomer Sepharose, moreover, the amount of ^{125}I -radioactivity bound to identical columns of fibrinogen-Sepharose and fibrin monomer-Sepharose did not differ significantly.

E. Effect of HA oligosaccharides on binding to fibrinogen

Initial studies indicated that there was a direct relationship between HA fragment size and fibrinogen binding affinity. We have further characterized this interaction and now know that an HA fragment with approximately 200 monosaccharide residues is as effective as high molecular weight native HA in eluting bound ^{125}I -fibrinogen from HA-Sepharose (Figure 1). Additionally, the amount of ^{125}I -fibrinogen bound by HA-Sepharose was directly related to the size of the HA-amine derivative attached to the resin.

F. HA-fibrin binding and its effect on fibrin polymerization and clot formation

We have proposed that the binding of HA to fibrin(ogen) could influence the structure of fibrin matrices and result in stabilization of the wound matrix. We have investigated this possibility by clotting fibrinogen with thrombin either in the presence or absence of HA. Clot formation is followed by monitoring the change in optical density (e.g. at 350 nm) over time. Results from these studies demonstrate that HA accelerates the rate of polymerization of fibrin monomers in a concentration dependent fashion. Additionally, fibrin matrices containing HA have a greater optical density than fibrin matrices without HA. If our results are comparable to earlier studies by others on fibrin gel formation, then the increased optical density of fibrin gels in the presence of HA would indicate a greater degree of lateral association between fibrin fibrils. This increased lateral association could result in a more stable fibrin matrix.

The significance of the above studies is accentuated by the recent finding by Gilula and his coworkers that fibrin is a normal component of the extracellular matrix. The interest in the results of this work, supported by this ONR contract, has been great judging by the number of reprint requests for the Journal of Theoretical Biology paper presenting the novel wound healing model.

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