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EVALUATION OF BIOCOMPATIBILITY OF CLINICAL IMPLANT MATERIALS USING DIGITAL IMAGES PROCESING

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Abstract. The use of computer vision coupled with scanning electron microscopy (SEM) was used to monitor the platelet adhesion and activation onto blood-contacting materials. The interaction of blood platelets with polyethylene (PE), poly(ethylene terephtalate (PET) and poly(vinylchloride) (PVC) after contact of the polymeric surfaces with whole blood was studied. The SEM images (SEM Phillips XL 30) were captured using IImages+ 1.97 computer vision systems. A library with a considerable number of acceptance or rejection of samples have been conceived and implemented. The obtained results make the developed computational vision system a promising tool for the evaluation of blood compatibility of biomaterials.

***Keywords:** computer vision, biomaterials, blood platelets, scanning electron microscopy, prosthetic devices.*

1. Introduction

The decline in surgical risk during recent decades has encouraged the development of more complex procedures for prosthetic implantation. The development of prosthetic devices were blood comes into contact with foreign surfaces has been severely restricted due to the platelet adhesion and activation at the prosthesis-blood interface which lead to thrombus formation (Ratner, 1993; Ishihara, 1993).

The extensive literature on the field of blood-compatible polymers includes the preparation and modification of polymers to achieve haemocompatibility as well as the development of methods to monitor blood-materials interactions and investigations of the complex process of thrombogenesis in general (Bruck, 1974; Suzuki et al, 1983; Ratner et al, 1987; Imanishi, 1994).

Platelet adhesion from blood on a solid surface is a problem of interest in a variety of biological, medical and technological processes (Hanker, et al., 1988; Williams, 1987, Driver et al, 1999). It occurs on all types of solid surfaces, even on surfaces which have the same kind of charge as the blood cell. The activation of adhered platelet serve as conditioning factor which determines the extent of subsequent thrombosis.

Several methods are currently available to counting adhered platelets onto polymeric surfaces (Gott, V.L. et al, 1971; Adams et al, 1980, 1983; Ratner et al, 1992; Beeker et al, 1994, Tamada et al, 1995). However, only by scanning electron microscopy is possible evaluate alterations on the morphology of the adhered blood platelets caused by the interactions with polymeric surfaces.

In the field of biomaterials, scanning electron microscopy (SEM) is widely used for surface roughness-topography analysis (Hoffman et al, 1983) or protein and cellular adhesion studies in implants (Ip et al, 1985). In this technique, a secondary electron emission caused by a focused electron beam is measured and spatially imaged. Because platelets are much smaller than other cells, the morphological observation and analysis of the adhered platelets by scanning electron microscopy (SEM) has a potential to give rise to error on the part of individual observers. Computational vision applied to the biological imaging techniques are assuming an increasingly important role in providing information on structure and physiological function at the cellular, tissue, organ and organism level (Cootes et al,1994; Ballard et al, 1982).

The purpose of this work was to develop a methodology of the blood compatibility analysis of cardiovascular prosthesis using computational vision for the morphological study of the adhered platelets. The following polymers were tested for the study of platelet adhesion and activation: poly(tetrafluoroethylene) (PTFE), poly(ethylene) (PE), and poly(vinylchloride) (PVC). These polymers have dominated the developments in total joint replacement surgery, vascular prostheses and blood bags, respectively.

2. Experimental

The platelet adhesion assay on the polymeric surfaces was evaluated by the open-static method with whole human blood (de Queiroz et al, 1997). The tests were performed by depositing $2x10^{-6}$ m^3 of fresh blood onto each of the five test surfaces. After contact times of 180 s, the surfaces were washed with saline under carefully controlled conditions to remove all blood components that did not adhere. After fixation with glutaraldeyde and sputter-coated with gold, the morphological changes of adhered platelets were performed using scanning electron microscopy (SEM). A SEM Phillips XL 30 was used to platelet image acquisition data. The morphology of the adhered platelets was obtained from five photographs of different surface areas (10^{-4} m^2) of the same sample. The SEM images were captured using HLimages++97 computer vision systems (HLIMAGE, 1997). The library with a considerable number of features extraction algorithms, used for pattern classification and image analysis as well as acceptance or rejection of samples was conceived and implemented. Since the image characteristics like as spatial resolution may be dependent of the gray-level, in this work it was used 256 gray levels to make the processing efficient.

3. Results and Discussion

Figure 1 shows the electron micrographs of adherent blood platelets on the PTFE (1-A), PE (1-B) and PVC (1-C) surfaces, respectively. It is frequently noted in biomedical literature that glass represents a strongly thrombogenic surface and a clot-inducing surface (Park et al, 1988). In this work, glass coverslip was used as reference for adhesion and maximum platelet activation surface (Fig. 1-D).

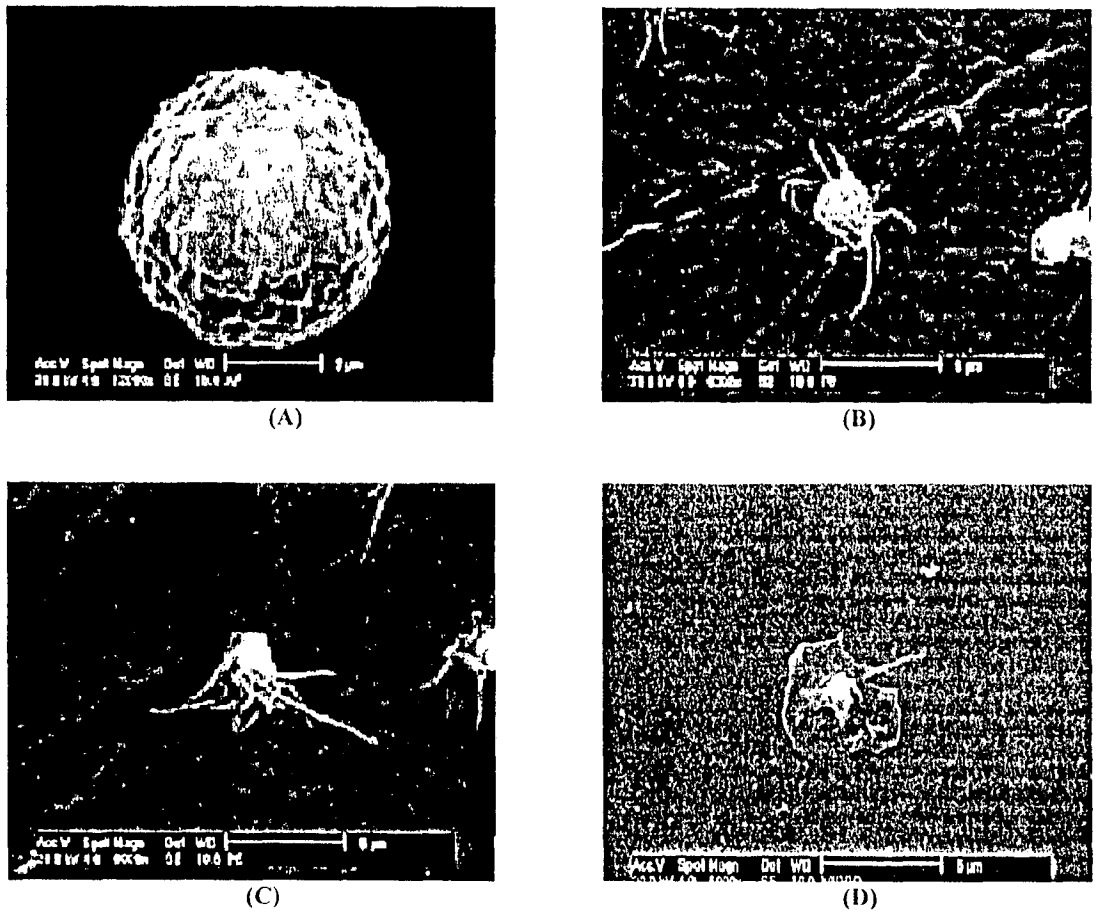


Figure 1. Scanning electron microscopic views of blood platelets adhered to the PTFE (A), PE (B), PVC (C) and Glass (D), respectively. Original magnifications of Figures x 1000. The bar indicate 5 μm.

The morphology of adherent platelets may be classified in according to the membrane cell activation and deformation. In this sense, adhered platelets without activation and aggregation with round morphology similar to that of native form may be classified as type I (Fig. 1-A). The blood platelets with a small degree of pseudopod extension was classified as type II (Fig. 1-B). Platelets with larger activation and spreading due to membrane rupture was classified as type III.

Its may be considered that Fig. 1A,D represents the extremes of the platelet morphology alteration. Thus, the platelet SEM images (Fig. 1-A,D) were captured from the scanning electron microscopy (SEM), using HLimages++97 computer vision systems and segmented. The image segmentation is a process of segmenting an image into a group of homogeneous regions according to whose characteristics such as color and texture. The segmentation results of the platelet type I and III are shown in Fig. 1-C and 2-B,C.

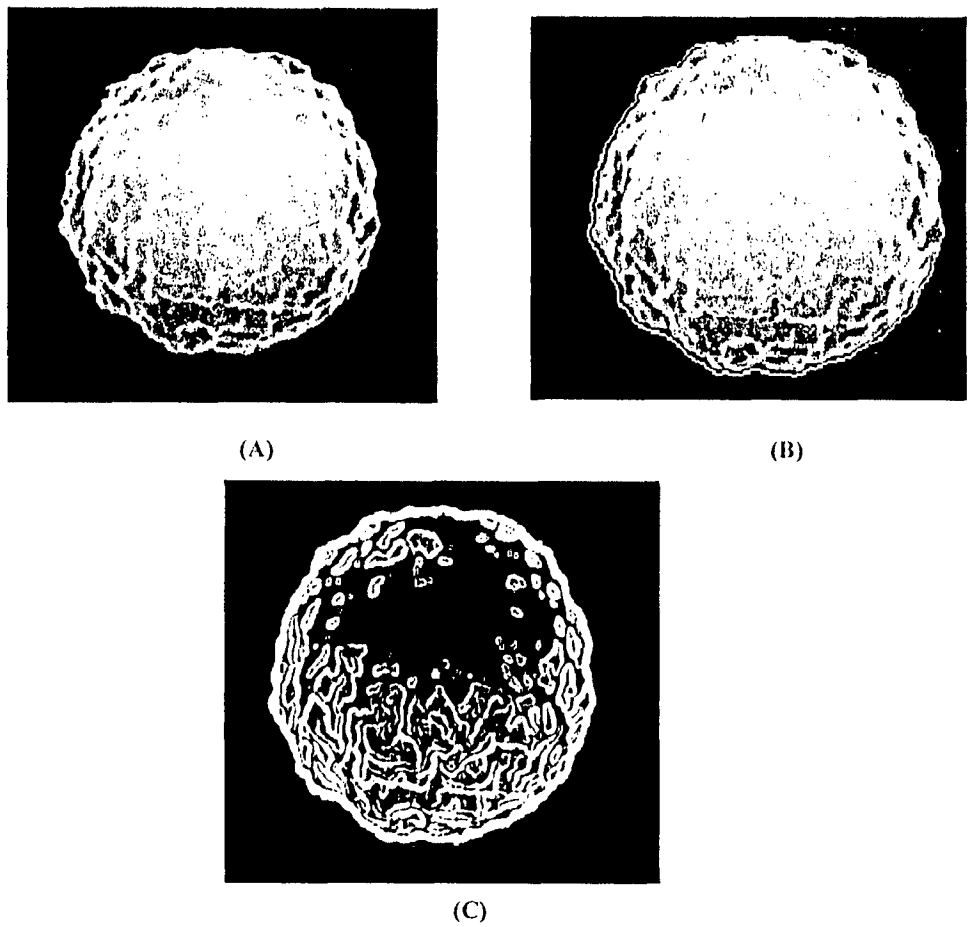


Figure 2. SEM micrograph of the platelet type I (A), segmented image (B) and binary image (C).

The digital image is preprocessed using technique that transform the image intensities in a spatial array of gray levels to remove large local discontinuities. Thus, a gray-level histogram of the image may be obtained. A gray-level histogram of an image is a function that gives the frequency of occurrence of each gray level in the image. The gray levels are quantified from 0 to n, the value of the histogram at a particular gray level p, denoted h(p), is the number or fraction of pixels in the image with that gray level. Each histogram of the images may be transformed in a binary image where one representative point of the reference image is contained in a window of n x n pixels.

Figure 4-A,B shows the histograms for the segmented Figures 2-3. Such histograms suggest that a high quality digitized platelet images are being localized at ranging of 100-150 of gray level.

As a first approximation, another features may be used in the image processing, such as the image compaction degree (perimeter^2/total area) or the rate between the total area and nucleus area for platelets type III. These are commonly measurements that through computational vision systems may be used for the morphology differentiation of blood platelets adhered on the biomaterials surface.

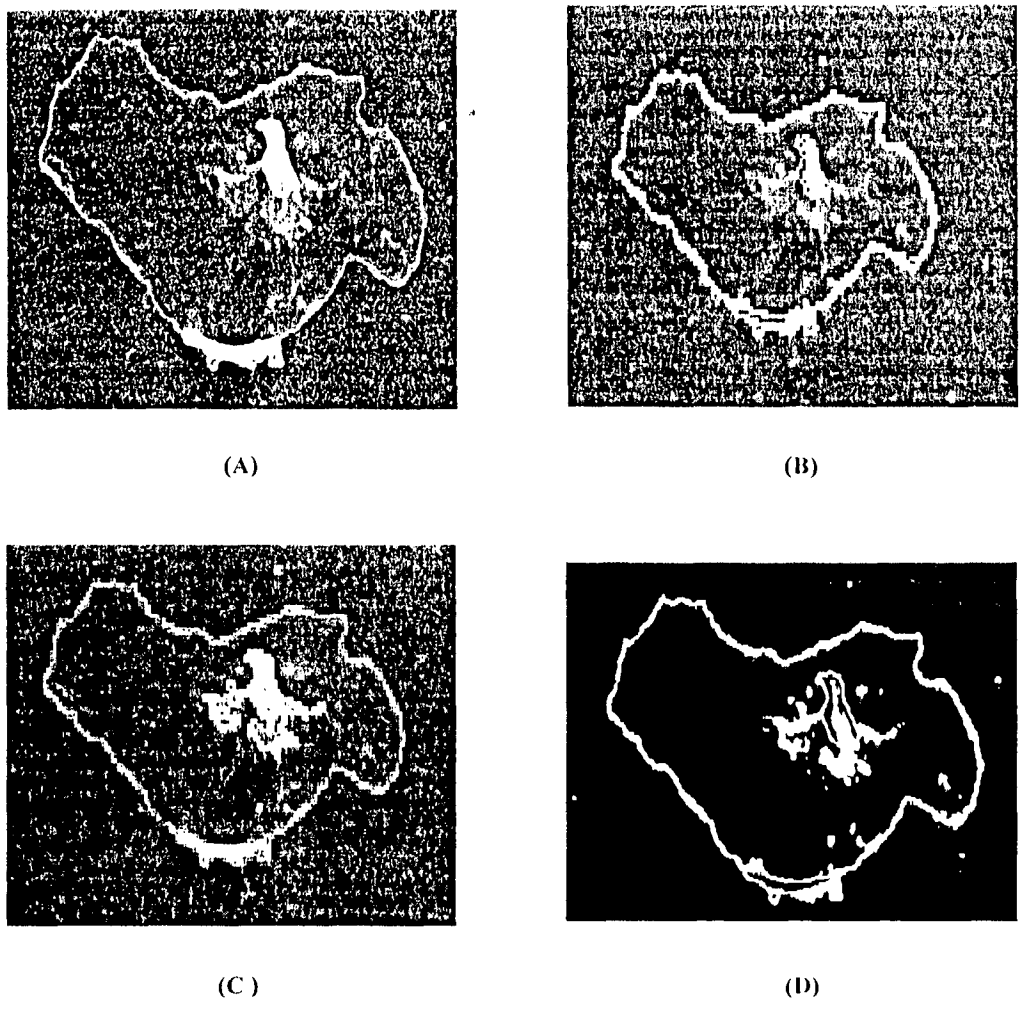
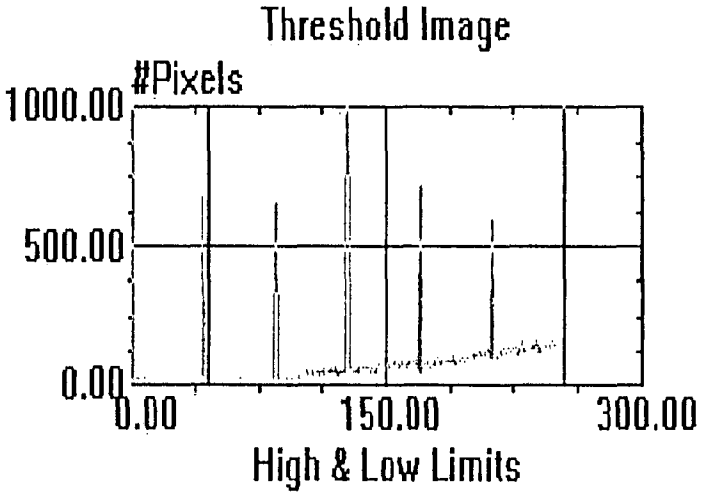
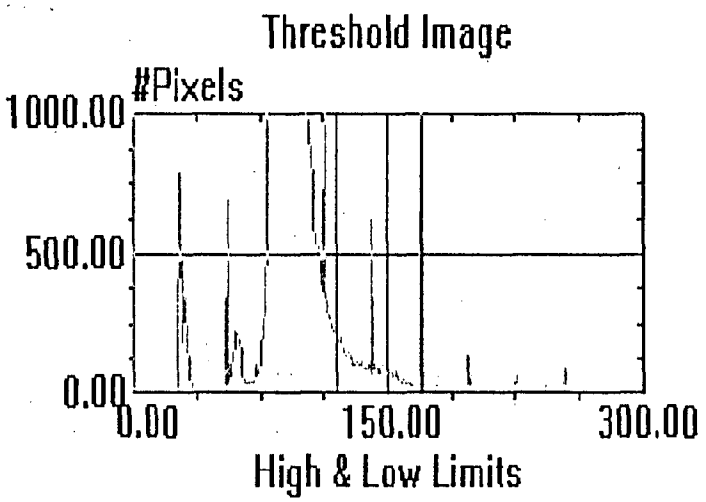


Figure 3. SEM micrograph of the blood platelet type III (A), segmented images (B,C) and binary image (D).



(A)



(B)

Figure 4. Intensity histogram of both segmented Figures 2 (A) and 3 (B), respectively.

4. Conclusions

There are many methods that we can bring to bear for the blood compatibility studies of cardiovascular prosthesis. Standard method include scanning electron microscopy or other conventional optical microscopes. However, an integration between biomaterials scientists and computation engineers is desirable to greatly enhance the information content available from the traditional methods. In fact, the computational vision supply valuable information's about the membrane activation stages of the adhered platelets on cardiovascular devices. In this work, computational vision using image segmentation, color and texture interpretation and others features-extraction of binary image were used for recognition and evaluation of the activated and nonactivated adhered platelets onto polymeric surfaces. In conclusion, it may be summarized that the computational vision is an effective technique for counting the adhered platelet as well as classify the activation stage of the membrane cells on to a synthetic surface. Consideration of other image parameters that may be used to define the platelets image such as image compaction degree (perimeter^2/total area) or the rate between the total area and nucleus will be used in the differentiation of the platelet activation processes. However, an extensive statistical treatment from the digitized images have been made to classify exactly the platelet activation stages. Measurements such as confidence limits and possibly regression calculations have been made. Without such indications, the obtained data may be meaningless.

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ABSTRACTS

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