Characterization of Bioactive Components in Human Blood Clot

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INTRODUCTION

In the past, several studies have demonstrated the role of platelet rich plasma (PRP) in enhancing tissue healing. PRP has been shown to contain growth factors essential to the natural healing process[1]. However, several shortcomings of PRP have been reported. PRP production is expensive, time consuming, and is inconsistent depending on the patient and the preparation method[2]. In addition most methods involve a chemical activation step that activates any latent growth factors, including TGF-β1. Lastly, PRP is a liquid that disseminates upon injection into the body prohibiting long term contact with the injured area.

An alternative method of preparing PRP is to form a blood clot using the ClotMaster Hula Cup device. The ClotMaster uses autologous whole blood, and by swirling the Hula cup for approximately 10 minutes the clotting cascade is initiated and a platelet rich clot is formed[3]. The clot produced can be used for long term direct delivery for growth factors and as a three dimensional native scaffold to which cells can adhere and proliferate. Our aim in this project was to assess the bioactivity of these clots produced in a much more efficient, economical manner than traditional PRP. To evaluate bioactivity, we used immunohistochemical stains to determine the presence of the growth factors essential to tissue healing including TGF-β1, PDGF-ββ, VEGF, and FGF.

METHODS

Figure 1. The ClotMaster hula cup

Following manufacturer's specifications, 20 mL of fresh human blood was drawn using a butterfly needle into a 60ml syringe. The blood was immediately transferred into the Hula Cup. The glass rod was positioned for a gel clot, and the cup was gently swirled for one minute. The blood was then allowed to stand stagnant for 9 minutes. After this procedure, the blood had formed a semi-solid gel clot. The clot was removed from the Hula Cup using forceps, and transferred to a container with unbuffered zinc formalin fixative solution. The gel clot was fixed for 48 hours at room temperature, embedded in paraffin, and sectioned.

We examined the morphology of the blood clots and assessed whether or not the desired growth factors were present. Using rabbit polyclonal antibodies from abcam, immunohistochemistry was performed to assess the presence of growth factors PDGF-ββ, TGF-β1, VEGF, and FGF basic. For each growth factor, we compared positive staining to a negative control.

RESULTS

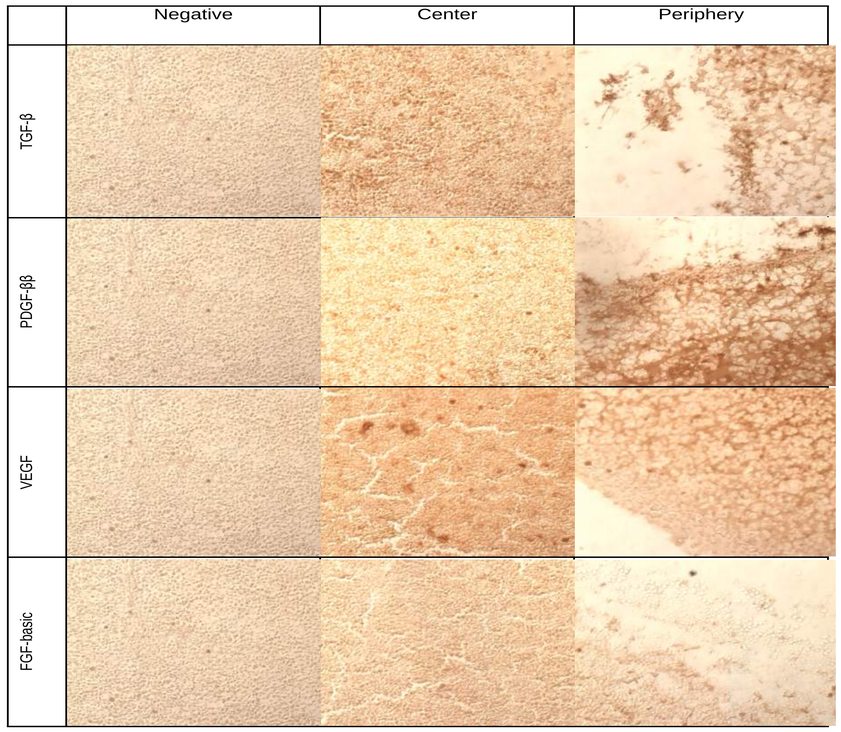

Figure 2. Immunohistochemical staining for growth factors

Four growth factors were tested. From top to bottom they were TGF-β, PDGF-ββ, VEGF, and FGF-2. The three columns from left to right represent negative controls, images of the center, and images of the periphery of the clot. Images were obtained using a Nikon Eclipse E800 light microscope at 40x magnification. Each clot was approximately 1 centimeter in diameter. A higher intensity of staining was found at the periphery for each growth factor. VEGF and TGF-β had a greater distribution of positive staining, while FGF-2 showed very little staining.

The results of this study show that growth factors essential to tissue healing are present in human blood clot produced by the Hula Cup. This indicates that blood clots will enhance tissue healing at least as well as PRP. As shown by the right column, there is a greater staining intensity around the periphery of the blood clot, where the blood made physical contact with the Hula cup, as compared to the center of the clot for all growth factors. In addition, there is a higher intensity of TGF-β and VEGF staining in the clots than PDGF-ββ and FGF-2. Further investigation is necessary to determine the distribution and quantity of these growth factors in not only the gel clot, but also the dense fibrous clot and the discoid membrane clot that can be made with the ClotMaster Hula Cup.

DISCUSSION

The positive staining for VEGF, TGF-β1, and PDGF-ββ indicates that clots produced by the ClotMaster Hula cup have viable tissue healing properties. The fact that these bioactive components are present shows that blood clots are capable of enhancing healing at least as well as traditional centrifuge derived Platelet Rich Plasma without the expensive, time consuming, and inconsistent preparation process. Given that Hula Cup clots have similar growth factor content to PRP, and are 3D scaffolds that can be made more economically, efficiently, and consistently, these clots are potentially superior tissue healing adjuvants. We chose to assess the presence of these particular growth factors to compare clots to PRP based on literature stating these as the significant growth factors found in traditional PRP [1,4]. IGF-1 and FGF-basic have also been found in PRP [4]. We plan to perform immunohistochemistry for these additional growth factors in the future.

The next steps to characterizing the tissue healing properties of clot will be to determine whether TGF-β found in the clot are is in its latent or active form. Another downside to traditional PRP is that chemical activation in the preparation process causes TGF-β to be activated [2]. This means it is activated prior to injection into the patient, which leads to a short period of drug delivery as the half-life of TGF-β is not long. If the clot contains TGF-β in latent form, it can be activated in the body causing a slower drug release, and therefore longer dosage to improve healing. Furthermore, quantification is underway for the amount of growth factors in the clot using ELISA assays.

In addition, we aim to better characterize the morphology of the clot through histological staining to visualize the distribution of platelets. We will assess the platelet capture rate as compared to PRP. In literature, the platelet capture rate of PRP ranges from 17-80% while Hula Cup blood clot platelet capture rate was found to be 92%[4],[5].

Finally, we will conduct a direct comparison of ClotMaster clots to traditional PRP by producing both in a controlled experiment using the same patient's blood on the same day to produce each type side by side. We plan to compare ClotMaster to the Cascade PRP preparation system because Cascade is the most commonly used brand in orthopedics, our research audience, and we want to compare to the current gold standard. We will assess the same variables discussed above for both.

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