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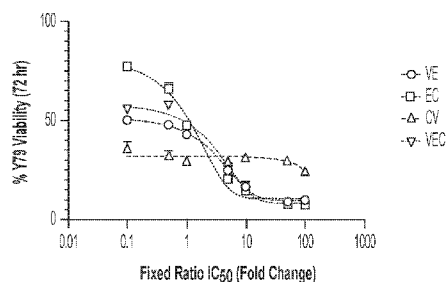


FIG. 2A

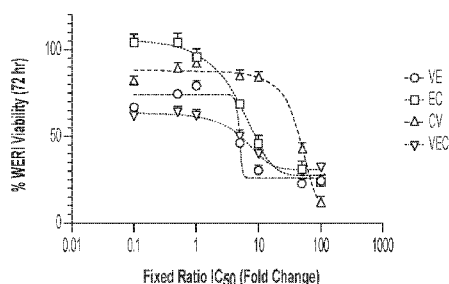


FIG. 2B

(57) Abstract: Methods and compositions for treating retinoblastoma are disclosed. The methods comprise administering a composition comprising two or more chemotherapeutic agents and a fibrin sealant material to a patient in need thereof. The compositions comprise two or more chemotherapeutic agents and a fibrin sealant material. Also disclosed are dual-compartment syringes comprising two or more chemotherapeutic agents, fibrinogen, and thrombin.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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SUBCONJUNCTIVAL CHEMOTHERAPEUTIC DELIVERY BY FIBRIN SEALANT IN THE TREATMENT OF RETINOBLASTOMA

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/810,681 filed February 26, 2019, U.S. Provisional Patent Application No. 62/925,639 filed October 24, 2019, and U.S. Provisional Patent Application No. 62/943,916 filed December 5, 2019. The entire content of each of the aforementioned applications is incorporated herein by reference for all purposes.

FIELD OF THE DISCLOSURE

[0002] The present invention relates to compositions and treatment methods for retinoblastoma.

BACKGROUND

[0003] Retinoblastoma is the most common intraocular cancer in children, accounting for roughly 9,000 new cases every year and claiming the lives of up to 70% of cases in certain regions of the world. While modern retinoblastoma treatments in developed countries have significantly reduced mortality rates, post-treatment consequences such as vision loss, off-site organ dysfunction, and secondary metastases remain unmet clinical needs. The clinical standard of care consistently uses systemic administration of a chemotherapeutic cocktail of vincristine, etoposide, and carboplatin (VEC). Clinical trials have demonstrated that subconjunctival delivery of aqueous chemotherapy is an effective method of tumor suppression, likely due to significant permeability across the scleral membrane and diffusion through the vitreous. While high intraocular drug levels can be achieved through this approach, rapid clearance results in poor efficacy.

[0004] Fibrin sealants are FDA-approved biodegradable tissue adhesives that have been used clinically for hemostasis and tissue sealing. More recently they have been used within the contexts of cellular, gene, and chemotherapeutic delivery systems. Studies have explored the use of fibrin sealants to promote carboplatin retention at the scleral surface and to minimize the injections regimen necessary for tumor management. However, such studies did not explore a VEC combinatorial approach using fibrin sealants. There exists a need for more efficacious retinoblastoma treatments, particularly treatments that avoid rapid clearance.

SUMMARY

[0005] The present disclosure provides compositions and methods for treating retinoblastoma. The compositions for treating retinoblastoma comprise two or more chemotherapeutic agents and a fibrin sealant material. The combination of two or more chemotherapeutic agents, such as the combination of vincristine, etoposide, and carboplatin (VEC), advantageously provides drug synergism.

[0006] The methods for treating retinoblastoma comprise administering a composition comprising two or more chemotherapeutic agents and a fibrin sealant material to a patient in need thereof.

[0007] The present disclosure also provides a dual-compartment syringe for administering the compositions disclosed herein according to the methods disclosed here. The dual-compartment syringe comprises two or more chemotherapeutic agents, fibrinogen, and thrombin.

[0008] An advantage of the drug matrix delivery system disclosed herein is that rapid drug clearance can be overcome.

[0009] A further advantage of the drug matrix delivery system disclosed herein is that a drug delivery system with fewer adverse effects compared with systemic delivery can be provided.

[0010] Yet another advantage of the disclosure is that fibrin polymerization (e.g., clot formation or structural attributes) is not appreciably affected by incorporation of etoposide and carboplatin in both whole blood and sealant formulations.

[0011] Still another advantage of the disclosure is that vincristine increases fibrin polymerization time without affecting overall clot strength (e.g., maximal clot amplitude or modulus).

[0012] An additional advantage of the disclosure is that compositional changes to the fibrin sealant can alter drug-matrix interactions, fluid permeability, and influence overall drug retention.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Figures 1A to 1F are graphs showing the inhibitory effect of cytotoxic compounds on Y79 and WERI retinoblastoma cells. Cell viability was assessed following a resazurin incubation and represented as a percentage to appropriate controls. Data are represented as mean $\pm$ SD (n=5-8).

[0014] Figures 2A and 2B are graphs showing inhibitory effect of VEC combination treatments on Y79 and WERI retinoblastoma cells.

[0015] Figure 3 depicts algorithms for computerized simulation of synergism, additivism and antagonism of the effect of multiple drugs.

[0016] Figure 4A is a dose reduction plot generated for WERI cell lines (n=6).

[0017] Figure 4B is a combination index plot generated for WERI cell lines (n=6).

[0018] Figure 4C is a dose reduction plot generated for Y79 cell lines (n=6).

[0019] Figure 4D is a combination index plot generated for Y79 cell lines (n=6).

[0020] Figure 5A shows representative TEG curves and quantified outputs.

[0021] Figure 5B shows a summary table of outputs normalized to the patient/sample control in whole blood (top) or fibrin sealant (bottom).

[0022] Figures 6A to 6D are graphs depicting thromboelastography (TEG) analysis of Chemotherapies in whole blood and fibrin sealant. Data are reported as fold changes from baseline controls $\pm$ SEM (n=5).

[0023] Figure 6E depicts changes in TEG parameters reported as percent change from baseline $\pm$ SEM (n=5).

[0024] Figures 7A to 7F depict fibrin polymerization rates in blood plasma and fibrin sealant with VEC supplementation. Purified or reconstituted thrombin [5 IU/mL]/CaCl₂ [0.025M] were supplemented with chemotherapies VEC (125-1000 uM) prior to triggering clot formation in plasma or reconstituted fibrinogen (3 mg/mL) in 96-well plates. Figures 7A to 7C indicate fibrin

formation rates over 40 minutes as determined 405 nm absorbance in blood plasma. Figures 7D to 7F indicate fibrin formation rates over 40 minutes as determined 405 nm absorbance in blood plasma fibrin sealants.

[0025] Figures 8A to 8D depict fiber analysis of VEC-embedded fibrin sealant. Commercially available fibrin sealant was admixed with 1 mM additions of Vincristine, Etoposide, or Carboplatin. Samples were critically point dried and imaged by scanning electron microscopy at 5,000X magnification at various points around the samples. Images were uploaded into ImageJ for fiber analysis. Figure 8A shows a grayscale 2-D fibrin image. In Figure 8B, the images were binarized to 8-bit grayscale. In Figure 8C, the binarized image was skeletonized to produce a representation of the fiber network. The Analyze Skeleton function was used to quantify fiber branches and junctions. Figure 8D shows a representative histogram output of branching numbers. Images analysed were taken at 5,000x magnification and compared over a 800 x 800 pixel area.

[0026] Figure 9 shows a plot of branch to junction ratios, where n=25-40 per treatment group. (*P<.05).

[0027] Figure 10 shows representative images of fiber structures by treatment group.

[0028] Figure 11 illustrates a representative embodiment of a dual-compartment syringe.

DETAILED DESCRIPTION

[0029] The present disclosure provides methods and compositions for treating retinoblastoma. The present disclosure also provides a dual-compartment syringe for delivering the disclosed compositions according to the disclosed methods.

Methods for Treating Retinoblastoma

[0030] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a method for treating retinoblastoma is provided comprising: administering a composition comprising two or more chemotherapeutic agents and a fibrin sealant material to a patient in need thereof.

[0031] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are selected from the group consisting of: vincristine, etoposide, carboplatin, melphalan, doxorubicin, fluorouracil, carmustine, methotrexate, and mixtures thereof.

[0032] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin.

[0033] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, each chemotherapeutic agent is independently present in the composition in a concentration of about 1 μ M to about 1 mM, such as about 10 μ M to about 1 mM, about 50 μ M to about 500 μ M, about 100 μ M to about 250 μ M, about 100 μ M to about 200 μ M, about 100 μ M to about 150 μ M, about 1 μ M to about 5 μ M, about 5 μ M to about 10 μ M, about 10 μ M to about 20 μ M, about 20 μ M to about 30 μ M, about 30 μ M to about 40 μ M, about 40 μ M to about 50 μ M, about 50 μ M to about 60 μ M, about 60 μ M to about 70 μ M, about 70 μ M to about 80 μ M, about 80 μ M to about 90 μ M, about 90 μ M to about 100 μ M, about 100 μ M to about 120 μ M, about 120 μ M to about 140 μ M, about 140 μ M to about 160 μ M, about 160 μ M to about 180 μ M, about 180 μ M to about 200 μ M, about 200 μ M to about 220 μ M, about 220 μ M to about 240 μ M, about 240 μ M to about 260 μ M, about 260 μ M to about 280 μ M, about 280 μ M to about 300 μ M, about 300 μ M to about 350 μ M, about 350 μ M to about 400 μ M, about 400 μ M to about 500 μ M, about 500 μ M to about 600 μ M, about 600 μ M to about 700 μ M, about 700 μ M to about 800 μ M, about 800 μ M to about 900 μ M, and/or about 900 μ M to about 1 mM.

[0034] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are present in the composition in a total concentration of about 3 μ M to about 3 mM, such as about 30 μ M to about 3 mM, about 150 μ M to about 1.5 mM, about 300 μ M to about 750 μ M, about 300 μ M to about 600 μ M, about 300 μ M to about 450 μ M, about 3 μ M to about 15 μ M, about 15 μ M to about 30 μ M, about 30 μ M to about 60 μ M, about 60 μ M to about 90 μ M, about 90 μ M to about 120 μ M, about 120 μ M to about 150 μ M, about 150 μ M to about 180 μ M, about 180 μ M to about

210 uM, about 210 uM to about 240 uM, about 240 uM to about 270 uM, about 270 uM to about 300 uM, about 300 uM to about 360 uM, about 360 uM to about 420 uM, about 420 uM to about 480 uM, about 480 uM to about 540 uM, about 540 uM to about 600 uM, about 600 uM to about 660 uM, about 660 uM to about 720 uM, about 720 uM to about 780 uM, about 780 uM to about 840 uM, about 840 uM to about 900 uM, about 900 uM to about 1050 uM, about 1050 uM to about 1200 uM, about 1200 uM to about 1.5 mM, about 1.5 mM to about 1.8 mM, about 1.8 mM to about 2.1 mM, about 2.1 mM to about 2.4 mM, about 2.4 mM to about 2.7 mM, and/or about 2.7 to about 3 mM.

[0035] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 0.05% to about 10% of the chemotherapeutical agents on a molar basis, such as about 0.05% to about 0.1%, about 0.1% to about 0.2%, about 0.2% to about 0.3%, about 0.3% to about 0.4%, about 0.4% to about 0.5%, about 0.5% to about 1%, about 1% to about 2%, about 2% to about 5%, and/or about 5% to about 10%.

[0036] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the etoposide is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0037] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture

of vincristine, etoposide, and carboplatin, and the carboplatin is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0038] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.05% to about 10% vincristine, about 10% to about 80% etoposide, and about 10% to about 80% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.1% to about 5% vincristine, about 15% to about 60% etoposide, and about 40% to about 80% carboplatin, about 0.1% to about 1% vincristine, about 20% to about 50% etoposide, and about 50% to about 80% carboplatin, about 0.1% to about 0.5% vincristine, about 25% to about 45% etoposide, and about 55% to about 75% carboplatin, about 0.1% to about 0.2% vincristine, about 30% to about 40% etoposide, and about 60% to about 70% carboplatin, and/or about 0.2% vincristine, about 34.9% etoposide, and about 64.9% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.7% vincristine, about 33.1% etoposide, and about 66.2% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for non-metastatic type retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:50:100 is used for treating non-metastatic type retinoblastomas. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.02% to about 1% vincristine, about 30% to about 90% etoposide, and about 10% to about 70% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.02% to about 0.5% vincristine, about 40% to about 80% etoposide, and about 20% to about 60% carboplatin, about 0.02% to about 0.1% vincristine, about 50% to about 70% etoposide, and about 25% to about 50% carboplatin, about 0.02% to about 0.05% vincristine,

about 50% to about 70% etoposide, and about 25% to about 45% carboplatin, and/or about 0.03% to about 0.04% vincristine, about 60% to about 70% etoposide, and about 30% to about 40% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.033% vincristine, about 66.644% etoposide, and about 33.322% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for metastatically-prone retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:2000:1000 is used for treating metastatically-prone retinoblastomas.

[0039] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises fibrinogen and thrombin.

[0040] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises fibrinogen in a concentration of about 10 mg/mL to about 200 mg/mL, such as about 30 mg/mL to about 150 mg/mL, about 50 mg/mL to about 120 mg/mL, about 75 mg/mL to about 125 mg/mL, about 80 mg/mL to about 100 mg/mL, about 90 mg/mL, about 10 mg/mL to about 30 mg/mL, about 30 mg/mL to about 50 mg/mL, about 50 mg/mL to about 70 mg/mL, about 70 mg/mL to about 90 mg/mL, about 90 mg/mL to about 110 mg/mL, about 110 mg/mL to about 130 mg/mL, about 130 mg/mL to about 150 mg/mL, about 150 mg/mL to about 170 mg/mL, and/or about 170 mg/mL to about 200 mg/mL.

[0041] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises thrombin in a concentration of about 10 IU/mL to about 2000 IU/mL, such as about 50 IU/mL to about 1000 IU/mL, about 100 IU/mL to about 800 IU/mL, about 250 IU/mL to about 750 IU/mL, about 300 IU/mL to about 700 IU/mL, about 400 IU/mL to about 600 IU/mL, and/or about 500 IU/mL.

[0042] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the composition is administered using a dual-compartment syringe.

[0043] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a first compartment of the syringe comprises the chemotherapeutic agents and thrombin, and a second compartment of the syringe comprises fibrinogen.

[0044] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a first compartment of the syringe comprises the chemotherapeutic agents and fibrinogen, and a second compartment of the syringe comprises thrombin.

[0045] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the composition is administered by subconjunctival injection.

Compositions for Treating Retinoblastoma

[0046] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a composition for treating retinoblastoma is provided comprising: two or more chemotherapeutic agents and a fibrin sealant material.

[0047] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are selected from the group consisting of: vincristine, etoposide, carboplatin, melphalan, doxorubicin, fluorouracil, carmustine, methotrexate, and mixtures thereof.

[0048] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin.

[0049] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, each chemotherapeutic agent is

independently present in the composition in a concentration of about 1 uM to about 1 mM, such as about 10 uM to about 1 mM, about 50 uM to about 500 uM, about 100 uM to about 250 uM, about 100 uM to about 200 uM, about 100 uM to about 150 uM, about 1 uM to about 5 uM, about 5 uM to about 10 uM, about 10 uM to about 20 uM, about 20 uM to about 30 uM, about 30 uM to about 40 uM, about 40 uM to about 50 uM, about 50 uM to about 60 uM, about 60 uM to about 70 uM, about 70 uM to about 80 uM, about 80 uM to about 90 uM, about 90 uM to about 100 uM, about 100 uM to about 120 uM, about 120 uM to about 140 uM, about 140 uM to about 160 uM, about 160 uM to about 180 uM, about 180 uM to about 200 uM, about 200 uM to about 220 uM, about 220 uM to about 240 uM, about 240 uM to about 260 uM, about 260 uM to about 280 uM, about 280 uM to about 300 uM, about 300 uM to about 350 uM, about 350 uM to about 400 uM, about 400 uM to about 500 uM, about 500 uM to about 600 uM, about 600 uM to about 700 uM, about 700 uM to about 800 uM, about 800 uM to about 900 uM, and/or about 900 uM to about 1 mM.

[0050] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are present in the composition in a total concentration of about 3 uM to about 3 mM, such as about 30 uM to about 3 mM, about 150 uM to about 1.5 mM, about 300 uM to about 750 uM, about 300 uM to about 600 uM, about 300 uM to about 450 uM, about 3 uM to about 15 uM, about 15 uM to about 30 uM, about 30 uM to about 60 uM, about 60 uM to about 90 uM, about 90 uM to about 120 uM, about 120 uM to about 150 uM, about 150 uM to about 180 uM, about 180 uM to about 210 uM, about 210 uM to about 240 uM, about 240 uM to about 270 uM, about 270 uM to about 300 uM, about 300 uM to about 360 uM, about 360 uM to about 420 uM, about 420 uM to about 480 uM, about 480 uM to about 540 uM, about 540 uM to about 600 uM, about 600 uM to about 660 uM, about 660 uM to about 720 uM, about 720 uM to about 780 uM, about 780 uM to about 840 uM, about 840 uM to about 900 uM, about 900 uM to about 1050 uM, about 1050 uM to about 1200 uM, about 1200 uM to about 1.5 mM, about 1.5 mM to about 1.8 mM, about 1.8 mM to about 2.1 mM, about 2.1 mM to about 2.4 mM, about 2.4 mM to about 2.7 mM, and/or about 2.7 to about 3 mM.

[0051] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture

of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 0.05% to about 10% of the chemotherapeutical agents on a molar basis, such as about 0.05% to about 0.1%, about 0.1% to about 0.2%, about 0.2% to about 0.3%, about 0.3% to about 0.4%, about 0.4% to about 0.5%, about 0.5% to about 1%, about 1% to about 2%, about 2% to about 5%, and/or about 5% to about 10%.

[0052] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the etoposide is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0053] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the carboplatin is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0054] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.05% to about 10% vincristine, about 10% to about 80% etoposide, and about 10% to about 80% carboplatin on a molar basis of the chemotherapeutical agents, such as about

0.1% to about 5% vincristine, about 15% to about 60% etoposide, and about 40% to about 80% carboplatin, about 0.1% to about 1% vincristine, about 20% to about 50% etoposide, and about 50% to about 80% carboplatin, about 0.1% to about 0.5% vincristine, about 25% to about 45% etoposide, and about 55% to about 75% carboplatin, about 0.1% to about 0.2% vincristine, about 30% to about 40% etoposide, and about 60% to about 70% carboplatin, and/or about 0.2% vincristine, about 34.9% etoposide, and about 64.9% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.7% vincristine, about 33.1% etoposide, and about 66.2% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for non-metastatic type retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:50:100 is used for treating non-metastatic type retinoblastomas. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.02% to about 1% vincristine, about 30% to about 90% etoposide, and about 10% to about 70% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.02% to about 0.5% vincristine, about 40% to about 80% etoposide, and about 20% to about 60% carboplatin, about 0.02% to about 0.1% vincristine, about 50% to about 70% etoposide, and about 25% to about 50% carboplatin, about 0.02% to about 0.05% vincristine, about 50% to about 70% etoposide, and about 25% to about 45% carboplatin, and/or about 0.03% to about 0.04% vincristine, about 60% to about 70% etoposide, and about 30% to about 40% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.033% vincristine, about 66.644% etoposide, and about 33.322% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for metastatically-prone retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:2000:1000 is used for treating metastatically-prone retinoblastomas.

[0055] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises fibrinogen and thrombin.

[0056] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises fibrinogen in a concentration of about 10 mg/mL to about 200 mg/mL, such as about 30 mg/mL to about 150 mg/mL, about 50 mg/mL to about 120 mg/mL, about 75 mg/mL to about 125 mg/mL, about 80 mg/mL to about 100 mg/mL, about 90 mg/mL, about 10 mg/mL to about 30 mg/mL, about 30 mg/mL to about 50 mg/mL, about 50 mg/mL to about 70 mg/mL, about 70 mg/mL to about 90 mg/mL, about 90 mg/mL to about 110 mg/mL, about 110 mg/mL to about 130 mg/mL, about 130 mg/mL to about 150 mg/mL, about 150 mg/mL to about 170 mg/mL, and/or about 170 mg/mL to about 200 mg/mL.

[0057] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material comprises thrombin in a concentration of about 10 IU/mL to about 2000 IU/mL, such as about 50 IU/mL to about 1000 IU/mL, about 100 IU/mL to about 800 IU/mL, about 250 IU/mL to about 750 IU/mL, about 300 IU/mL to about 700 IU/mL, about 400 IU/mL to about 600 IU/mL, and/or about 500 IU/mL.

[0058] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material is obtained by combining a first solution comprising the chemotherapeutical agents and thrombin with a second solution comprising fibrinogen.

[0059] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrin sealant material is obtained by combining a first solution comprising fibrinogen with a second solution comprising the chemotherapeutical agents and thrombin.

Dual-Compartment Syringes

[0060] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a dual-compartment syringe is provided comprising two or more chemotherapeutic agents, fibrinogen, and thrombin.

[0061] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a first compartment of the syringe comprises the chemotherapeutic agents and thrombin, and a second compartment of the syringe comprises fibrinogen.

[0062] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents and thrombin are present as a solution, and the fibrinogen is present as a solution.

[0063] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the solutions are frozen.

[0064] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents and thrombin are present as a dry powder, and the fibrinogen is present as a dry powder.

[0065] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the dry powders are reconstituted to form a solution.

[0066] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, a first compartment of the syringe comprises the chemotherapeutic agents and fibrinogen, and a second compartment of the syringe comprises thrombin.

[0067] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are selected from the group consisting of: vincristine, etoposide, carboplatin, melphalan, doxorubicin, fluorouracil, carmustine, methotrexate, and mixtures thereof.

[0068] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin.

[0069] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, each chemotherapeutic agent is independently present in the composition in a concentration of about 1 uM to about 1 mM, such as about 10 uM to about 1 mM, about 50 uM to about 500 uM, about 100 uM to about 250 uM, about 100 uM to about 200 uM, about 100 uM to about 150 uM, about 1 uM to about 5 uM, about 5 uM to about 10 uM, about 10 uM to about 20 uM, about 20 uM to about 30 uM, about 30 uM to about 40 uM, about 40 uM to about 50 uM, about 50 uM to about 60 uM, about 60 uM to about 70 uM, about 70 uM to about 80 uM, about 80 uM to about 90 uM, about 90 uM to about 100 uM, about 100 uM to about 120 uM, about 120 uM to about 140 uM, about 140 uM to about 160 uM, about 160 uM to about 180 uM, about 180 uM to about 200 uM, about 200 uM to about 220 uM, about 220 uM to about 240 uM, about 240 uM to about 260 uM, about 260 uM to about 280 uM, about 280 uM to about 300 uM, about 300 uM to about 350 uM, about 350 uM to about 400 uM, about 400 uM to about 500 uM, about 500 uM to about 600 uM, about 600 uM to about 700 uM, about 700 uM to about 800 uM, about 800 uM to about 900 uM, and/or about 900 uM to about 1 mM.

[0070] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are present in the composition in a total concentration of about 3 uM to about 3 mM, such as about 30 uM to about 3 mM, about 150 uM to about 1.5 mM, about 300 uM to about 750 uM, about 300 uM to about 600 uM, about 300 uM to about 450 uM, about 3 uM to about 15 uM, about 15 uM to about 30 uM, about 30 uM to about 60 uM, about 60 uM to about 90 uM, about 90 uM to about 120 uM, about 120 uM to about 150 uM, about 150 uM to about 180 uM, about 180 uM to about 210 uM, about 210 uM to about 240 uM, about 240 uM to about 270 uM, about 270 uM to about 300 uM, about 300 uM to about 360 uM, about 360 uM to about 420 uM, about 420 uM to about 480 uM, about 480 uM to about 540 uM, about 540 uM to about 600 uM, about 600 uM to about 660 uM, about 660 uM to about 720 uM, about 720 uM to about 780 uM, about 780 uM to about 840 uM, about 840 uM to about 900 uM, about 900 uM to about 1050 uM, about 1050 uM to

about 1200 uM, about 1200 uM to about 1.5 mM, about 1.5 mM to about 1.8 mM, about 1.8 mM to about 2.1 mM, about 2.1 mM to about 2.4 mM, about 2.4 mM to about 2.7 mM, and/or about 2.7 to about 3 mM.

[0071] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 0.05% to about 10% of the chemotherapeutical agents on a molar basis, such as about 0.05% to about 0.1%, about 0.1% to about 0.2%, about 0.2% to about 0.3%, about 0.3% to about 0.4%, about 0.4% to about 0.5%, about 0.5% to about 1%, about 1% to about 2%, about 2% to about 5%, and/or about 5% to about 10%.

[0072] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the etoposide is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0073] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the carboplatin is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

[0074] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.05% to about 10% vincristine, about 10% to about 80% etoposide, and about 10% to about 80% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.1% to about 5% vincristine, about 15% to about 60% etoposide, and about 40% to about 80% carboplatin, about 0.1% to about 1% vincristine, about 20% to about 50% etoposide, and about 50% to about 80% carboplatin, about 0.1% to about 0.5% vincristine, about 25% to about 45% etoposide, and about 55% to about 75% carboplatin, about 0.1% to about 0.2% vincristine, about 30% to about 40% etoposide, and about 60% to about 70% carboplatin, and/or about 0.2% vincristine, about 34.9% etoposide, and about 64.9% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.7% vincristine, about 33.1% etoposide, and about 66.2% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for non-metastatic type retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:50:100 is used for treating non-metastatic type retinoblastomas. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.02% to about 1% vincristine, about 30% to about 90% etoposide, and about 10% to about 70% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.02% to about 0.5% vincristine, about 40% to about 80% etoposide, and about 20% to about 60% carboplatin, about 0.02% to about 0.1% vincristine, about 50% to about 70% etoposide, and about 25% to about 50% carboplatin, about 0.02% to about 0.05% vincristine, about 50% to about 70% etoposide, and about 25% to about 45% carboplatin, and/or about 0.03% to about 0.04% vincristine, about 60% to about 70% etoposide, and about 30% to about 40% carboplatin. In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in

an amount of about 0.033% vincristine, about 66.644% etoposide, and about 33.322% carboplatin on a molar basis of the chemotherapeutical agents. In some cases, these chemotherapeutic mixtures are used treating for metastatically-prone retinoblastomas. For example, in some cases, a chemotherapeutic mixture of vincristine, etoposide, and carboplatin in a molar ratio of about 1:2000:1000 is used for treating metastatically-prone retinoblastomas.

[0075] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the fibrinogen is present in a concentration of about 10 mg/mL to about 200 mg/mL, such as about 30 mg/mL to about 150 mg/mL, about 50 mg/mL to about 120 mg/mL, about 75 mg/mL to about 125 mg/mL, about 80 mg/mL to about 100 mg/mL, about 90 mg/mL, about 10 mg/mL to about 30 mg/mL, about 30 mg/mL to about 50 mg/mL, about 50 mg/mL to about 70 mg/mL, about 70 mg/mL to about 90 mg/mL, about 90 mg/mL to about 110 mg/mL, about 110 mg/mL to about 130 mg/mL, about 130 mg/mL to about 150 mg/mL, about 150 mg/mL to about 170 mg/mL, and/or about 170 mg/mL to about 200 mg/mL.

[0076] In an embodiment of the present disclosure, which may be combined with any other embodiment listed herein unless specified otherwise, the thrombin is present in a concentration of about 10 IU/mL to about 2000 IU/mL, such as about 50 IU/mL to about 1000 IU/mL, about 100 IU/mL to about 800 IU/mL, about 250 IU/mL to about 750 IU/mL, about 300 IU/mL to about 700 IU/mL, about 400 IU/mL to about 600 IU/mL, and/or about 500 IU/mL.

[0077] As illustrated in FIG. 11, dual-compartment syringe 100 includes an applicator tip having a Y-connector 200 and a dispensing needle 204 that is coupled to and projects outward from the Y-connector 200.

[0078] The invention is further described in the examples below, yet without being restricted thereto.

EXAMPLES

[0079] Example 1 - Methods

[0080] Cell Culture

[0081] The human retinoblastoma Y79 (ATCC® HTB18™) and WERI (ATCC® HTB169™) cell lines were cultured in RPMI 1640 (ATCC®) medium supplemented with 20% (Y79) or 10% (WERI-RB) fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere of 5% CO₂ at 37°C, grown in 75 cm² tissue culture flasks. Once confluent, cells were sub cultivated with fresh medium 1:2 to 1:4 ratios after discarding the supernatant and obtaining the gravity-settled aggregates.

[0082] Cell Viability assays

[0083] 10 mL of cell suspension were centrifuged at 1000 G for 10 minutes. The supernatant was discarded and the pellet was then resuspended in media to 1.1×10^5 live cells/mL following a viability cell count check. Cells were plated in 96-well plates at 10,000 cells per well. Sample wells were treated with carboplatin, etoposide or vincristine at the concentrations of 1000 uM, 100 uM, 50 uM, 10 uM, 5 uM, 1 uM, 0.5 uM, and 0.1 uM. Untreated and non-cell controls include a 10 uL vehicle, bringing up all wells to 100 uL total volume. Experiments covered 24/48/72 hour incubations at 5% CO₂ at 37°C prior to analyses. Plates ready for analysis were incubated with 10 uL of 10X Alamar Blue Reagent for 1-4 hours (apparent color change) and emission intensity read at 585 nm following a 555 nm excitation. The results are shown in FIG. 1.

[0084] Y79 and WERI cells were incubated with Vincristine, Etoposide, or Carboplatin using previously established half-maximal inhibitory concentrations in a fixed-ratio treatment paradigm. Y79 and WERI cells also were incubated with a mixture of Vincristine, Etoposide, or Carboplatin. Cell viability was assessed following a resazurin incubation and represented as a percentage to appropriate Y79 and WERI controls, as shown in FIGs. 2A and 2B, respectively.

[0085] Combination Analyses

[0086] The combination effects of VEC were analyzed using combination index (CI) and dose reduction index (DRI) analyses. Plots were generated using CompuSyn software employing the Chou-Talalay calculation methods of determining drug synergism as depicted in FIG. 3. Representative plots are shown in FIGs. 4A to 4D.

[0087] Transwell Viability Assays

[0088] Fibrin sealant molds (0.5 mL) were prepared in 12 mm transwell inserts using 5 IU/mL thrombin admixed with fixed molar VEC ratios previously estimated upon the half maximal inhibitory concentrations of Y79 and WERI cell lines. Ratios were tested at 0.1, 0.5, 1, 50, and 100-fold concentrations. The discs were incubated for 72 hours in 1.5 mL full growth media with Y79 or WERI (2.2×10^5 live cells/mL). After 72 hours, wells were incubated with Alamar Blue Reagent for 1-4 hours (apparent color change) and emission intensity read at 585 nm following a 555 nm excitation. Dose-response curves for each drug were generated and the inhibitory concentration values extrapolated.

[0089] Thromboelastography Analysis of Chemotherapies in Whole Blood and Fibrin Glue

[0090] Alterations in the clot strength and formation kinetics were determined in whole blood and fibrin sealant following additions of Vincristine, Etoposide, or Carboplatin.

[0091] Clot formation kinetics and strength were determined with thromboelastography (TEG) using Thromboelastograph® Hemostasis Analyzer Model 5000 (Haemonetics Co.) with the following deviations. The initial set of experiments were prepared according to standard TEG operating procedures, testing for alterations in the clotting properties following additions of chemotherapeutic Vincristine, Etoposide, and Carboplatin compared to a saline or DMSO controls. In short, fifty-six (56 uL) of a CaCl_2 [0.011M]/ chemotherapy [1 mM] solution were placed in cups wells prior to the addition of 304 uL of whole blood for a total well volume of 360 uL. The TEG analyses were started at $t=15$ seconds for all sets of samples and run at 37°C.

[0092] An additional set of experiments were performed using TISSEEL™ fibrin glue, using reconstituted fibrinogen and thrombin mixtures to generate a clot. To begin, 304 uL of reconstituted fibrinogen protein concentrate was diluted to physiological plasma concentrations (~3 mg/mL) and placed in the cup wells. Fifty-six (56 uL) of reconstituted thrombin [5 IU/mL]/ chemotherapy [1 mM] were then added, bringing the total well volume of 360 uL. The TEG analyses were started at $t=15$ seconds for all sets of samples and run at 37°C.

[0093] TEG Analytical Software version 4.2.3 was used to calculate the time to clot initiation time (R, minutes), time to clot firmness (K, minutes), alpha angle (α , degrees), maximal clot strength (MA, maximum amplitude, mm), and shear elastic modulus strength (G, dynes/cm²).

Analyses were performed for at least 30 minutes or until the operating software had calculated all mentioned parameters. The results are shown in FIG. 5 and FIGs. 6A to 6E.

[0094] The effects of VEC on fibrin formation were also determined from adapted densitometry or thromboelastography (TEG) techniques. Fibrin monomer formation was monitored (A405 nm) in VEC-supplemented (0.5-1 mM) plasma or fibrinogen concentrate following clot generation by calcium or thrombin. Viscoelastic properties of clot formation were determined in VEC-supplemented whole blood or fibrinogen concentrate triggered by thrombin.

[0095] TEG Analytical Software version 4.2.3 was used to calculate the time to clot initiation (R, minutes), time to clot firmness (K, minutes), alpha angle (α , degrees), maximal clot strength (MA, maximum amplitude, mm), and shear elastic modulus strength (G, dynes/cm²).

[0096] Fibrinokinetics

[0097] Citrated pool blood plasma was obtained from Loyola blood bank. Chemotherapeutics Vincristine, Etoposide, and Carboplatin were reconstituted in PBS or small concentrations of DMSO. Fibrinokinetics were measured in microtiter plates using 175 microliter of plasma supplemented with 25 uL drug [1 mM-0.25 mM], and using a solution of 50 uL of purified thrombin [5 IU/mL]/CaCl₂ [0.025M] to a trigger clot generation. For the control well plates 25uL of DMSO or PBS was added. The rate of clot formation was monitored for 40 minutes at A405 nm. Both kinetic and endpoint methods were used to calculate the rate of clot formation.

[0098] TISSEEL™ fibrinogen protein concentrate was diluted to physiological plasma concentrations (~3 mg/mL). Chemotherapeutics Vincristine, Etoposide, and Carboplatin were reconstituted in PBS or small concentrations of DMSO. Fibrinokinetics were measured in microtiter plates using 175 microliter of plasma supplemented with 25 uL drug [1 mM-0.25 mM], and using a solution of 50 uL of purified thrombin [5 IU/mL]/CaCl₂ [0.025M] to a trigger clot generation. For the control well plates 25uL of DMSO or PBS was added. The rate of clot formation was monitored for 40 minutes at A405 nm. Both kinetic and endpoint methods were used to calculate the rate of clot formation. The results are provided in FIG. 7.

[0099] Static Dialysis

[00100] Three fibrinogen and thrombin protein vials were reconstituted at 37 degrees. Following reconstitution, the thrombin solutions were diluted with reconstituted doxorubicin in water to bring the final thrombin concentration of three 0.5 mL samples containing 5 IU/mL, 100 IU/mL, or 500 IU/mL; each containing 7.5 mM Doxorubicin HCl (1.88 grams).

[00101] Following solution preparation, thrombin and fibrinogen solutions, each solution was pulled up into respective syringes and loaded in a Duploject spray system and sprayed into 300 uL PELCO 105 molds and allowed to coagulate for 1 minutes at room temperature. The molds were then placed in 5cm × 10mm pre-soaked and rinsed Spectra/Por Biotech regenerated cellulose dialysis membrane tubing (Spectrum Laboratories, MWCO: 3.5–5 kDa), and the suspension dialyzed at 37°C by immersion in 20 ml PBS (pH 7.4) containing 0.05% NaN₃ with continuous gentle stirring. At timed intervals, a 50 uL aliquot of dialysate was removed and replaced with an equal volume of PBS. Doxorubicin present in the dialysate was quantified by HPLC as described above, with an external standard curve prepared in water.

[00102] To determine the actual delivered drug concentrations, two molds were dissolved by 0.05% trypsin at 37 degrees Celsius, centrifuged at 21,000 g to remove most of the protein content, and the supernatant verified by HPLC.

[00103] Generating HPLC Standard Curves

[00104] Chemotherapy drugs were resolved by HPLC (Hitachi LaChrom Elite, model 2200) using a Beckman Coulter Ultrasphere 5 µm C18 reverse-phase column (4.6 mm × 25 cm). An isocratic mobile phase of acetonitrile and formic acid (0.05N) was used to elute each respective drug from the column at a rate of 1 ml/min. Table 1 provides the mobile phase and detection parameters used for each drug standard created.

Table 1

Drug	% Formic Acid (0.05N)	% Acetonitrile	Wavelength Detection	Retention Time (min)
Carboplatin	98.7	1.3	230	4.39
Carmustine	30	70	230	3.39
Doxorubicin	70	30	233	4.92
Etoposide	30	70	280	3.37

[00105] Method development occurred following literature searches for HPLC parameters and instrumentation used for each drug. A variety of acetonitrile:formic acid ratios were attempted for each drug until a satisfactory peak was resolved. Max absorption wavelengths were determined using a NanoDrop2000 (ThermoFisher Scientific) and were used as UV-Vis detection wavelength. Standard curves were generated for each drug, by creating known drug standards between (0.1-1 ug/uL). Solutions were run in triplicate, the areas under the detected drug peaks integrated, and linear fits were generated.

[00106] Scanning Electron Microscopy

[00107] TISSEEL™ or TISSEEL™ admixed with chemotherapeutic drug (1mM final concentration) was prepared according to the IFU. In short, 2 mM additions of chemotherapy were transferred via syringe and needle to the thrombin vial (500 IU/mL thrombin) during preparation and placed in a Fibrinotherm to spin and heat the mixtures to 37 degrees celsius. Following complete dissolution of both thrombin and fibrinogen solutions, each solution was pulled up into respective syringes and loaded in the Duploject spray system accompanying the kit. The 1 mL volumes of thrombin and fibrinogen (2 mL total) were sprayed onto collagen casing. Immediately following coagulation (~30 seconds), the samples were washed 3X in cacodylate buffer for 10 minutes and fixed in 2% glutaraldehyde buffer (Electron Microscopy Sciences). Fixed clots were washed, serially dehydrated with ethanol, and dried with hexamethyldisilazane (Electron Microscopy Sciences). Clots were allowed to air dry overnight thereafter. Clots were then teased apart with forceps and mounted on aluminum sample supports with carbon adhesive tape. Samples were sputter coated with 20 nm iridium metal in a Leica ACE600 and analyzed by scanning electron microscopy (FEI Quanta 650 FEG). The results are provided in FIGs. 8 and 10.

[00108] Branching and Pore Size Analysis

[00109] Branching analysis was performed using 2D images cross-sectional cuts of fibrin clots. Representative images and sections displaying easily discernible fibrin branching characteristics were selected from each group. Images were processed using ImageJ's skeleton analysis function. In short, images were first binarized to delineate the image further into branch and background sections. Binary images were then placed through the Analyze Skeleton

(2D/3D) function with no pruning or selected parameters. The output file included details such as branch lengths, branch numbers, and number of junctions. The results are provided in FIG. 9 and Table 2.

Table 2

	Max Branch Number	Junctions	Avg Branch length (nm)	Branch/Junction Ratio
Control	5043 +/- 108	71 +/- 1.5	26	71.0
Vincristine	7580 +/- 125	136 +/- 2.3	22	55.7
Etoposide	8272 +/- 140	125 +/- 2.1	21	66.2
Carboplatin	10065 +/- 142	148 +/- 2.6	19	68.0

[00110] Example 2 - Results

[00111] Standalone IC₅₀'s calculated for Vincristine, Etoposide, or Carboplatin in WERI cells were 2.9 uM, 2.7 uM, 1.0 uM; or in Y79 cells were 0.1 uM, 6.0 uM, and 1.1 uM respectively. Fixed-ratio VEC 72-hour combination treatments in WERI cells suggest synergistic effects at sub-micromolar concentrations, but antagonistic half maximal concentrations for any drugs alone. Fibrin polymerization and viscoelastic clot properties were unaffected by incorporation of etoposide and carboplatin in both whole blood and sealant formulations. Alternatively, vincristine impeded fibrin formation time, but did not affect maximal clot amplitude or modulus. These results were recapitulated by EM network analysis, in which branch-to-junction ratios significantly were significantly lower in V-embedded fibrin matrices (55.7) compared to saline controls (71.0).

[00112] Combined treatments performed antagonistically at levels needed to effectively inhibit tumor growth (>90%). Deviations in fibrin clot formation characteristics (as seen with high-dose 1 mM vincristine supplementation) may play a role VEC release rates and clot breakdown in vivo.

WHAT IS CLAIMED IS:

1. A method for treating retinoblastoma comprising: administering a composition comprising two or more chemotherapeutic agents and a fibrin sealant material to a patient in need thereof.
2. The method of claim 1, wherein the chemotherapeutic agents are selected from the group consisting of: vincristine, etoposide, carboplatin, melphalan, doxorubicin, fluorouracil, carmustine, methotrexate, and mixtures thereof.
3. The method of claim 1, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin.
4. The method of claim 1, wherein each chemotherapeutic agent is independently present in the composition in a concentration of about 1 uM to about 1 mM, such as about 10 uM to about 1 mM, about 50 uM to about 500 uM, about 100 uM to about 250 uM, about 100 uM to about 200 uM, about 100 uM to about 150 uM, about 1 uM to about 5 uM, about 5 uM to about 10 uM, about 10 uM to about 20 uM, about 20 uM to about 30 uM, about 30 uM to about 40 uM, about 40 uM to about 50 uM, about 50 uM to about 60 uM, about 60 uM to about 70 uM, about 70 uM to about 80 uM, about 80 uM to about 90 uM, about 90 uM to about 100 uM, about 100 uM to about 120 uM, about 120 uM to about 140 uM, about 140 uM to about 160 uM, about 160 uM to about 180 uM, about 180 uM to about 200 uM, about 200 uM to about 220 uM, about 220 uM to about 240 uM, about 240 uM to about 260 uM, about 260 uM to about 280 uM, about 280 uM to about 300 uM, about 300 uM to about 350 uM, about 350 uM to about 400 uM, about 400 uM to about 500 uM, about 500 uM to about 600 uM, about 600 uM to about 700 uM, about 700 uM to about 800 uM, about 800 uM to about 900 uM, and/or about 900 uM to about 1 mM.
5. The method of claim 1, wherein the chemotherapeutic agents are present in the composition in a total concentration of about 3 uM to about 3 mM, such as about 30 uM to about 3 mM, about 150 uM to about 1.5 mM, about 300 uM to about 750 uM, about 300 uM to about 600 uM, about 300 uM to about 450 uM, about 3 uM to about 15 uM, about 15 uM to about 30 uM, about 30 uM to about 60 uM, about 60 uM to about

90 uM, about 90 uM to about 120 uM, about 120 uM to about 150 uM, about 150 uM to about 180 uM, about 180 uM to about 210 uM, about 210 uM to about 240 uM, about 240 uM to about 270 uM, about 270 uM to about 300 uM, about 300 uM to about 360 uM, about 360 uM to about 420 uM, about 420 uM to about 480 uM, about 480 uM to about 540 uM, about 540 uM to about 600 uM, about 600 uM to about 660 uM, about 660 uM to about 720 uM, about 720 uM to about 780 uM, about 780 uM to about 840 uM, about 840 uM to about 900 uM, about 900 uM to about 1050 uM, about 1050 uM to about 1200 uM, about 1200 uM to about 1.5 mM, about 1.5 mM to about 1.8 mM, about 1.8 mM to about 2.1 mM, about 2.1 mM to about 2.4 mM, about 2.4 mM to about 2.7 mM, and/or about 2.7 to about 3 mM.

6. The method of claim 1, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%; or the vincristine is present in an amount of about 0.05% to about 10% of the chemotherapeutical agents on a molar basis, such as about 0.05% to about 0.1%, about 0.1% to about 0.2%, about 0.2% to about 0.3%, about 0.3% to about 0.4%, about 0.4% to about 0.5%, about 0.5% to about 1%, about 1% to about 2%, about 2% to about 5%, and/or about 5% to about 10%.

7. The method of claim 1, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the etoposide is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

8. The method of claim 1, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the carboplatin is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as

about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

9. The method of claim 1, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.05% to about 10% vincristine, about 10% to about 80% etoposide, and about 10% to about 80% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.1% to about 5% vincristine, about 15% to about 60% etoposide, and about 40% to about 80% carboplatin, about 0.1% to about 1% vincristine, about 20% to about 50% etoposide, and about 50% to about 80% carboplatin, about 0.1% to about 0.5% vincristine, about 25% to about 45% etoposide, and about 55% to about 75% carboplatin, about 0.1% to about 0.2% vincristine, about 30% to about 40% etoposide, and about 60% to about 70% carboplatin, and/or about 0.2% vincristine, about 34.9% etoposide, and about 64.9% carboplatin.

10. The method of claim 1, wherein the fibrin sealant material comprises fibrinogen and thrombin.

11. The method of claim 1, wherein the fibrin sealant material comprises fibrinogen in a concentration of about 10 mg/mL to about 200 mg/mL, such as about 30 mg/mL to about 150 mg/mL, about 50 mg/mL to about 120 mg/mL, about 75 mg/mL to about 125 mg/mL, about 80 mg/mL to about 100 mg/mL, about 90 mg/mL, about 10 mg/mL to about 30 mg/mL, about 30 mg/mL to about 50 mg/mL, about 50 mg/mL to about 70 mg/mL, about 70 mg/mL to about 90 mg/mL, about 90 mg/mL to about 110 mg/mL, about 110 mg/mL to about 130 mg/mL, about 130 mg/mL to about 150 mg/mL, about 150 mg/mL to about 170 mg/mL, and/or about 170 mg/mL to about 200 mg/mL.

12. The method of claim 1, wherein the fibrin sealant material comprises thrombin in a concentration of about 10 IU/mL to about 2000 IU/mL, such as about 50 IU/mL to about 1000 IU/mL, about 100 IU/mL to about 800 IU/mL, about 250 IU/mL to about 750 IU/mL, about 300 IU/mL to about 700 IU/mL, about 400 IU/mL to about 600 IU/mL, and/or about 500 IU/mL.

13. The method of claim 1 wherein the composition is administered using a dual-compartment syringe.

14. The method of claim 13, wherein a first compartment of the syringe comprises the chemotherapeutic agents and thrombin, and a second compartment of the syringe comprises fibrinogen.

15. The method of claim 13, wherein a first compartment of the syringe comprises the chemotherapeutic agents and fibrinogen, and a second compartment of the syringe comprises thrombin.

16. The method of claim 1, wherein the composition is administered by subconjunctival injection.

17. A composition for treating retinoblastoma comprising: two or more chemotherapeutic agents and a fibrin sealant material.

18. The composition of claim 17, wherein the chemotherapeutic agents are selected from the group consisting of: vincristine, etoposide, carboplatin, melphalan, doxorubicin, fluorouracil, carmustine, methotrexate, and mixtures thereof.

19. The composition of claim 17, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin.

20. The composition of claim 17, wherein each chemotherapeutic agent is independently present in the composition in a concentration of about 1 uM to about 1 mM, such as about 10 uM to about 1 mM, about 50 uM to about 500 uM, about 100 uM to about 250 uM, about 100 uM to about 200 uM, about 100 uM to about 150 uM, about 1 uM to about 5 uM, about 5 uM to about 10 uM, about 10 uM to about 20 uM, about 20 uM to about 30 uM, about 30 uM to about 40 uM, about 40 uM to about 50 uM, about 50 uM to about 60 uM, about 60 uM to about 70 uM, about 70 uM to about 80 uM, about 80 uM to about 90 uM, about 90 uM to about 100 uM, about 100 uM to about 120 uM, about 120 uM to about 140 uM, about 140 uM to about 160 uM, about 160 uM to about 180 uM, about 180 uM to about 200 uM, about 200 uM to about 220 uM, about

220 uM to about 240 uM, about 240 uM to about 260 uM, about 260 uM to about 280 uM, about 280 uM to about 300 uM, about 300 uM to about 350 uM, about 350 uM to about 400 uM, about 400 uM to about 500 uM, about 500 uM to about 600 uM, about 600 uM to about 700 uM, about 700 uM to about 800 uM, about 800 uM to about 900 uM, and/or about 900 uM to about 1 mM.

21. The composition of claim 17, wherein the chemotherapeutic agents are present in the composition in a total concentration of about 3 uM to about 3 mM, such as about 30 uM to about 3 mM, about 150 uM to about 1.5 mM, about 300 uM to about 750 uM, about 300 uM to about 600 uM, about 300 uM to about 450 uM, about 3 uM to about 15 uM, about 15 uM to about 30 uM, about 30 uM to about 60 uM, about 60 uM to about 90 uM, about 90 uM to about 120 uM, about 120 uM to about 150 uM, about 150 uM to about 180 uM, about 180 uM to about 210 uM, about 210 uM to about 240 uM, about 240 uM to about 270 uM, about 270 uM to about 300 uM, about 300 uM to about 360 uM, about 360 uM to about 420 uM, about 420 uM to about 480 uM, about 480 uM to about 540 uM, about 540 uM to about 600 uM, about 600 uM to about 660 uM, about 660 uM to about 720 uM, about 720 uM to about 780 uM, about 780 uM to about 840 uM, about 840 uM to about 900 uM, about 900 uM to about 1050 uM, about 1050 uM to about 1200 uM, about 1200 uM to about 1.5 mM, about 1.5 mM to about 1.8 mM, about 1.8 mM to about 2.1 mM, about 2.1 mM to about 2.4 mM, about 2.4 mM to about 2.7 mM, and/or about 2.7 to about 3 mM.

22. The composition of claim 17, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the vincristine is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%; or the vincristine is present in an amount of about 0.05% to about 10% of the chemotherapeutical agents on a molar basis, such as about 0.05% to about 0.1%, about 0.1% to about 0.2%, about 0.2% to about 0.3%, about 0.3% to about 0.4%, about 0.4% to about 0.5%, about 0.5% to about 1%, about 1% to about 2%, about 2% to about 5%, and/or about 5% to about 10%..

23. The composition of claim 17, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the etoposide is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

24. The composition of claim 17, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the carboplatin is present in an amount of about 10% to about 90% of the chemotherapeutical agents on a molar basis, such as about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, and/or about 80% to about 90%.

25. The composition of claim 17, wherein the chemotherapeutic agents are a mixture of vincristine, etoposide, and carboplatin, and the chemotherapeutic agents are present in an amount of about 0.05% to about 10% vincristine, about 10% to about 80% etoposide, and about 10% to about 80% carboplatin on a molar basis of the chemotherapeutical agents, such as about 0.1% to about 5% vincristine, about 15% to about 60% etoposide, and about 40% to about 80% carboplatin, about 0.1% to about 1% vincristine, about 20% to about 50% etoposide, and about 50% to about 80% carboplatin, about 0.1% to about 0.5% vincristine, about 25% to about 45% etoposide, and about 55% to about 75% carboplatin, about 0.1% to about 0.2% vincristine, about 30% to about 40% etoposide, and about 60% to about 70% carboplatin, and/or about 0.2% vincristine, about 34.9% etoposide, and about 64.9% carboplatin.

26. The composition of claim 17, wherein the fibrin sealant material comprises fibrinogen and thrombin.

27. The composition of claim 17, wherein the fibrin sealant material comprises fibrinogen in a concentration of about 10 mg/mL to about 200 mg/mL, such as about 30 mg/mL to about 150 mg/mL, about 50 mg/mL to about 120 mg/mL, about 75 mg/mL to about 125 mg/mL, about 80 mg/mL to about 100 mg/mL, about 90

mg/mL, about 10 mg/mL to about 30 mg/mL, about 30 mg/mL to about 50 mg/mL, about 50 mg/mL to about 70 mg/mL, about 70 mg/mL to about 90 mg/mL, about 90 mg/mL to about 110 mg/mL, about 110 mg/mL to about 130 mg/mL, about 130 mg/mL to about 150 mg/mL, about 150 mg/mL to about 170 mg/mL, and/or about 170 mg/mL to about 200 mg/mL.

28. The composition of claim 17, wherein the fibrin sealant material comprises thrombin in a concentration of about 10 IU/mL to about 2000 IU/mL, such as about 50 IU/mL to about 1000 IU/mL, about 100 IU/mL to about 800 IU/mL, about 250 IU/mL to about 750 IU/mL, about 300 IU/mL to about 700 IU/mL, about 400 IU/mL to about 600 IU/mL, and/or about 500 IU/mL.

29. The composition of claim 17, wherein the fibrin sealant material is obtained by combining a first solution comprising the chemotherapeutic agents and thrombin with a second solution comprising fibrinogen.

30. The composition of claim 17, wherein the fibrin sealant material is obtained by combining a first solution comprising fibrinogen with a second solution comprising the chemotherapeutic agents and thrombin.

31. A dual-compartment syringe comprising two or more chemotherapeutic agents, fibrinogen, and thrombin.

32. The dual-compartment syringe of claim 31, wherein a first compartment of the syringe comprises the chemotherapeutic agents and thrombin, and a second compartment of the syringe comprises fibrinogen.

33. The dual-compartment syringe of claim 32, wherein the chemotherapeutic agents and thrombin are present as a solution, and the fibrinogen is present as a solution.

34. The dual-compartment syringe of claim 33, wherein the solutions are frozen.

35. The dual-compartment syringe of claim 32, wherein the chemotherapeutic agents and thrombin are present as a dry powder, and the fibrinogen is present as a dry

powder.

36. The dual-compartment syringe of claim 35, wherein the dry powders are reconstituted to form a solution.

37. The dual-compartment syringe of claim 31, wherein a first compartment of the syringe comprises the chemotherapeutic agents and fibrinogen, and a second compartment of the syringe comprises thrombin.

38. The dual-compartment syringe of claim 37, wherein the chemotherapeutic agents and thrombin are present as a solution, and the fibrinogen is present as a solution.

39. The dual-compartment syringe of claim 38, wherein the solutions are frozen.

40. The dual-compartment syringe of claim 37, wherein the chemotherapeutic agents and thrombin are present as a dry powder, and the fibrinogen is present as a dry powder.

41. The dual-compartment syringe of claim 40, wherein the dry powders are reconstituted to form a solution.

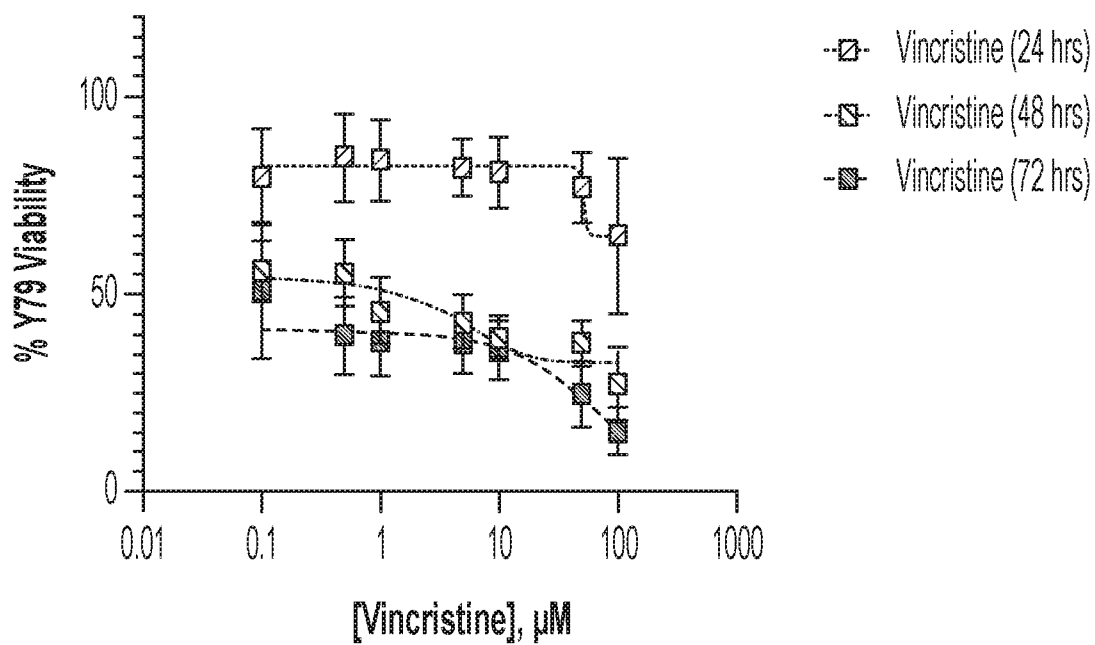


FIG. 1A

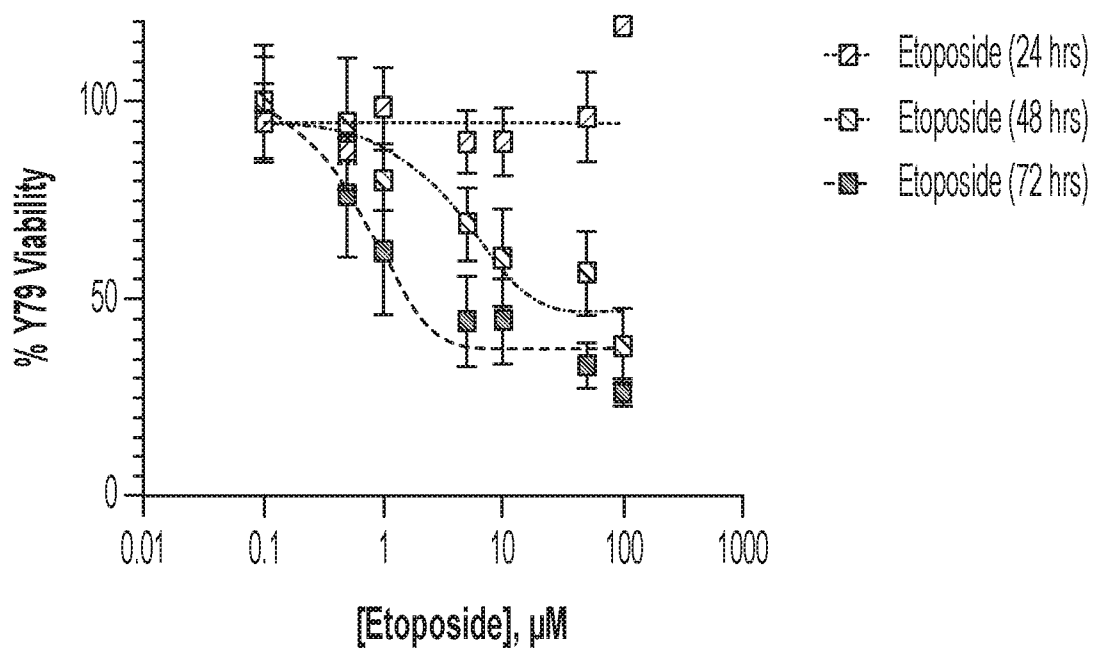


FIG. 1B

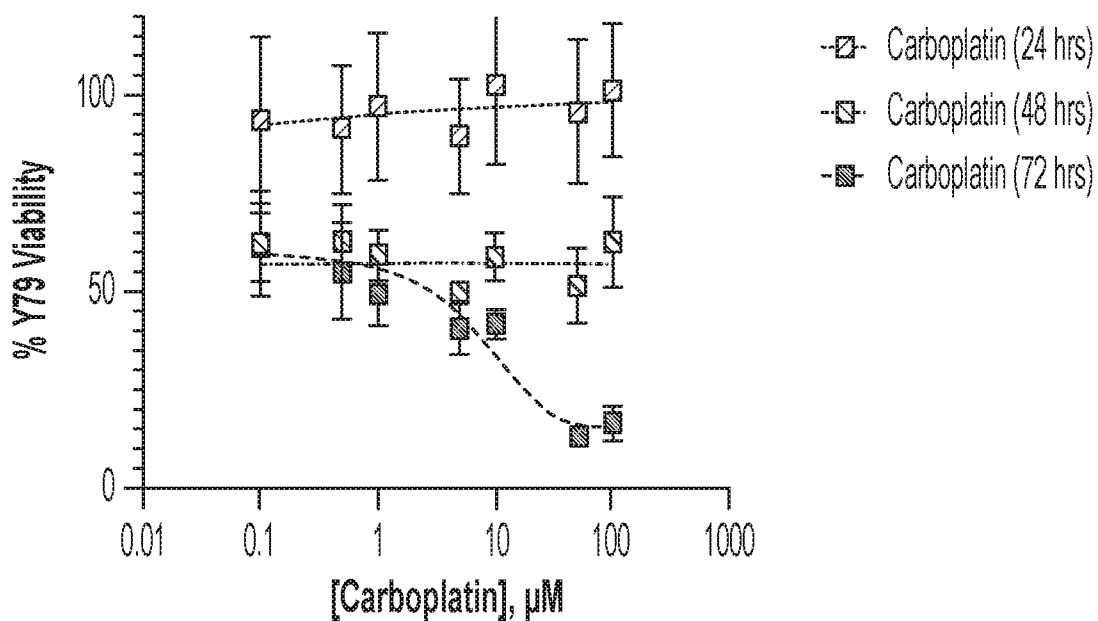


FIG. 1C

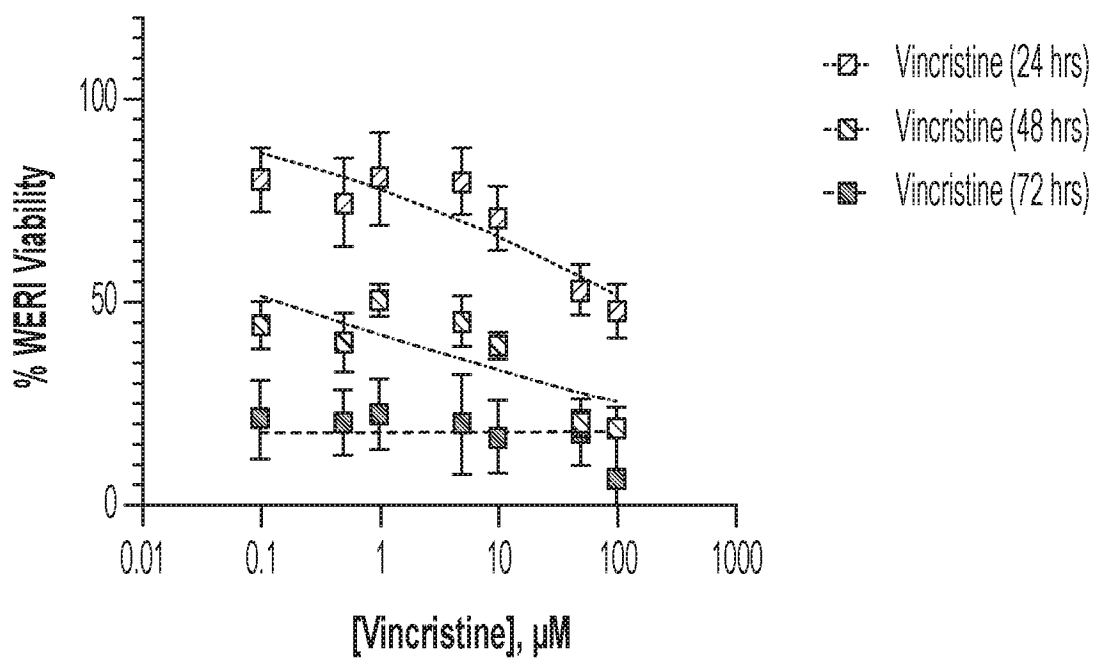


FIG. 1D

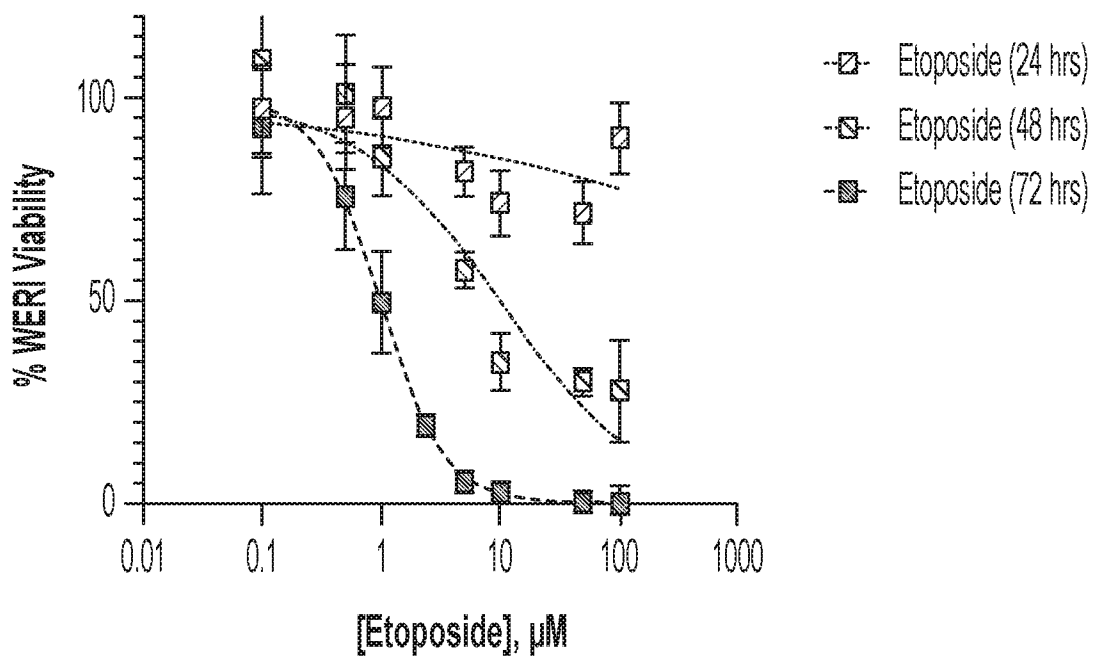


FIG. 1E

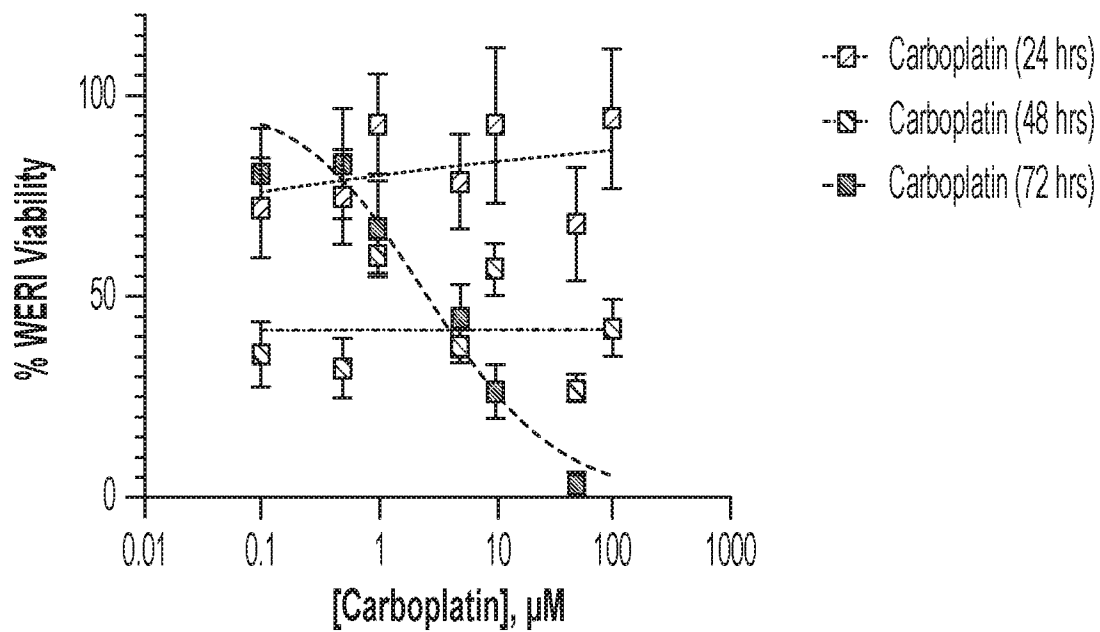


FIG. 1F

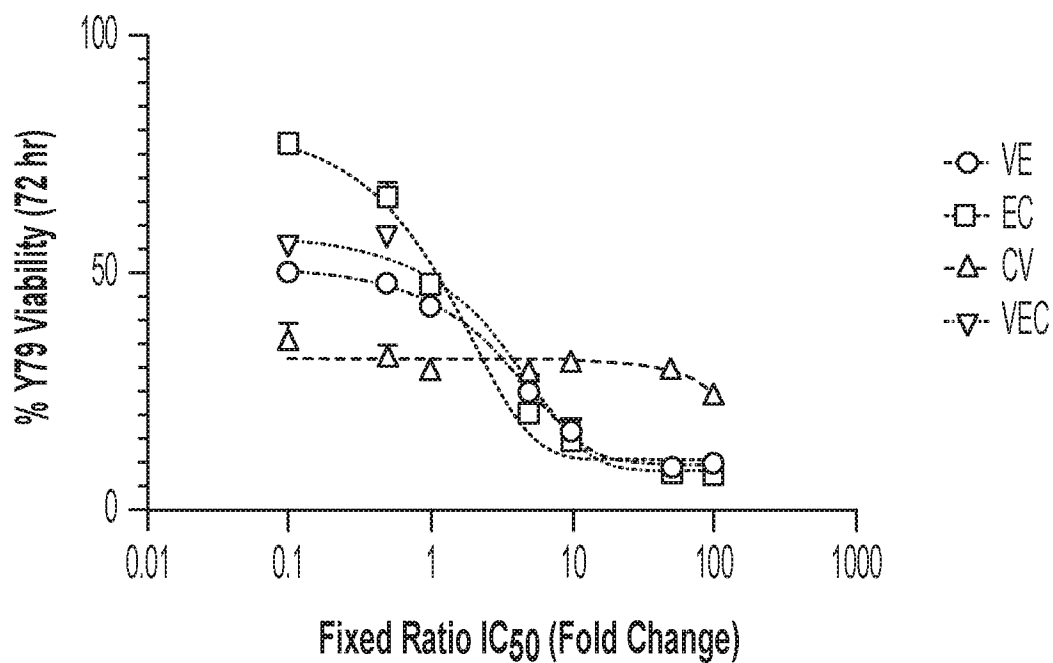


FIG. 2A

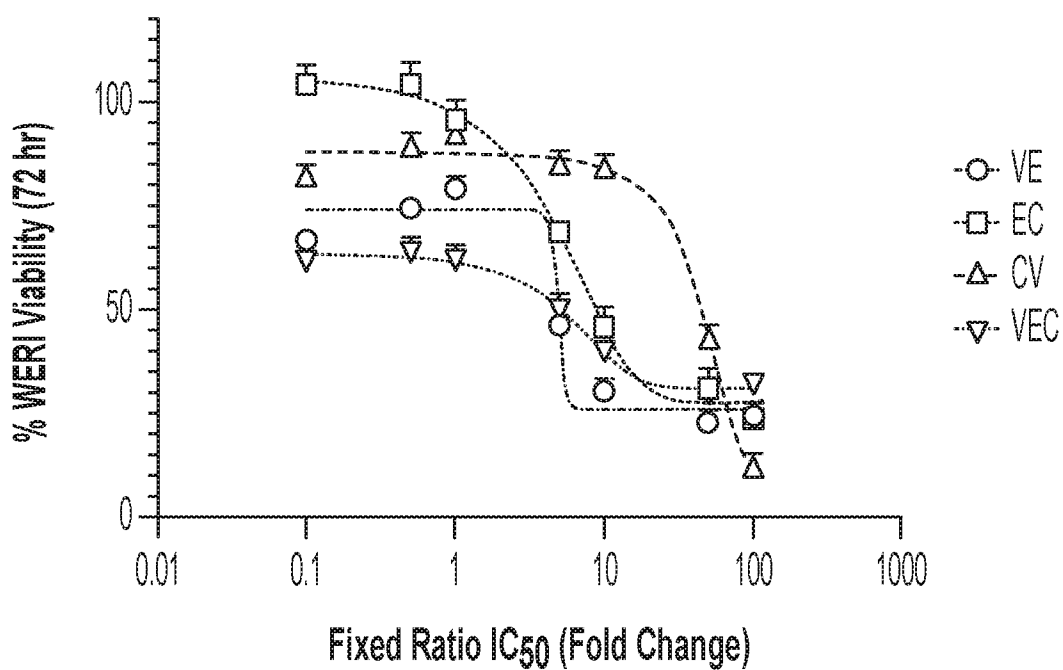


FIG. 2B

Algorithms for computerized simulation of synergism, additivism and antagonism of the effect of multiple drugs

The median-effect equation

$$E(1) f_a/f_u = (D/D_m)^m$$

$$E(2) \log(f_a/f_u) = m \log(D) - m \log(D_m)$$

$$E(3) f_a = 1/[1 + (D_m/D)^m]$$

$$E(4) D_x = D_m [f_a/(1-f_a)]^{1/m}$$

D = Dose

f_a = fraction affected

f_u = fraction unaffected

D_m = median-effect dose

m = slope, or kinetic order

$$\left[\text{For } n \text{ drug combinations: } CI = \sum_{j=1}^n \frac{(D)_j}{(D_x)_j} \right]$$

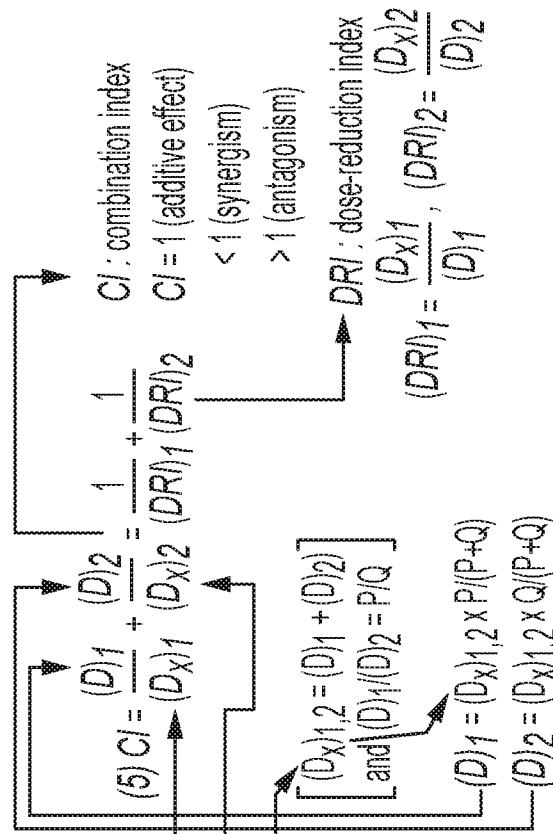


FIG. 3

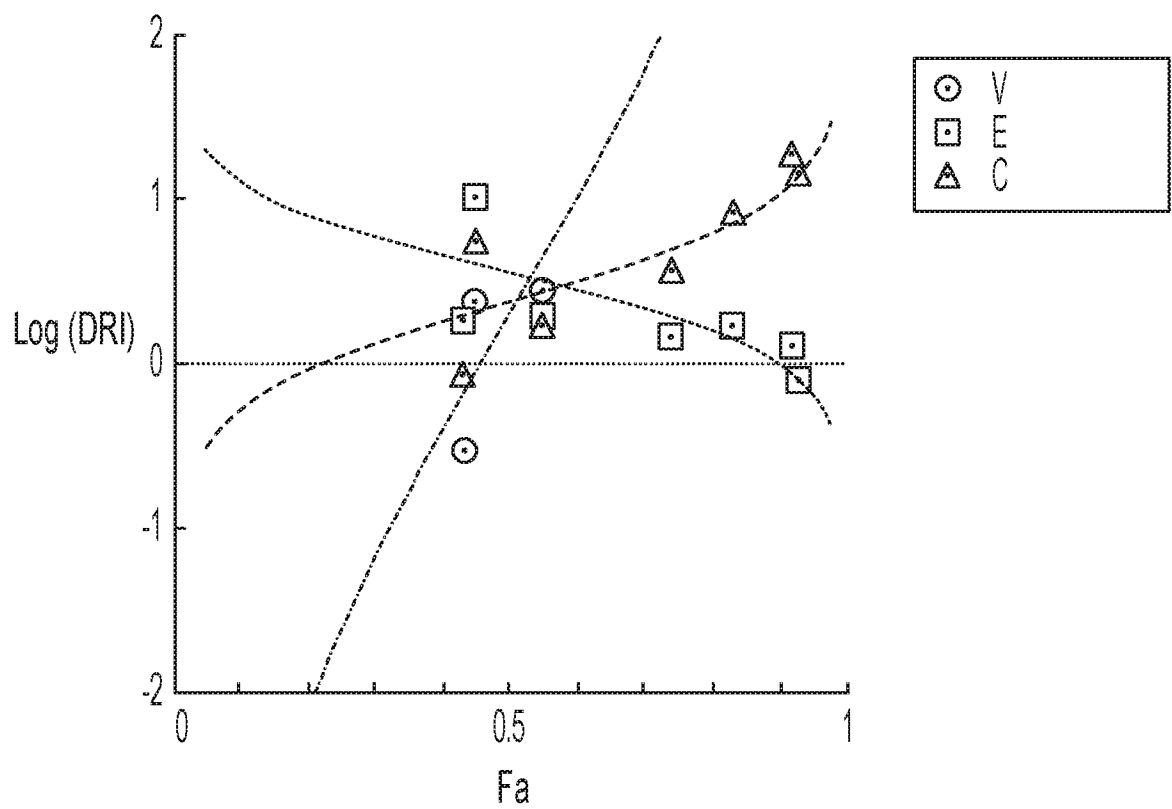


FIG. 4A

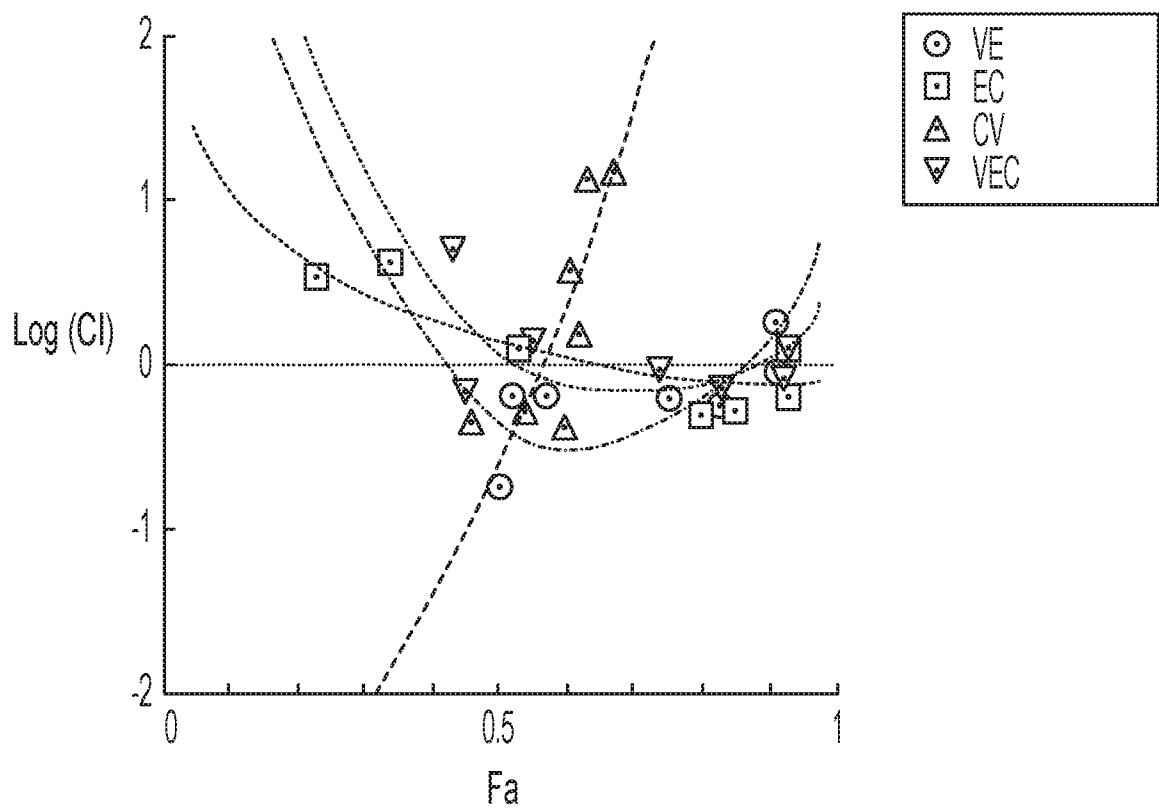


FIG. 4B

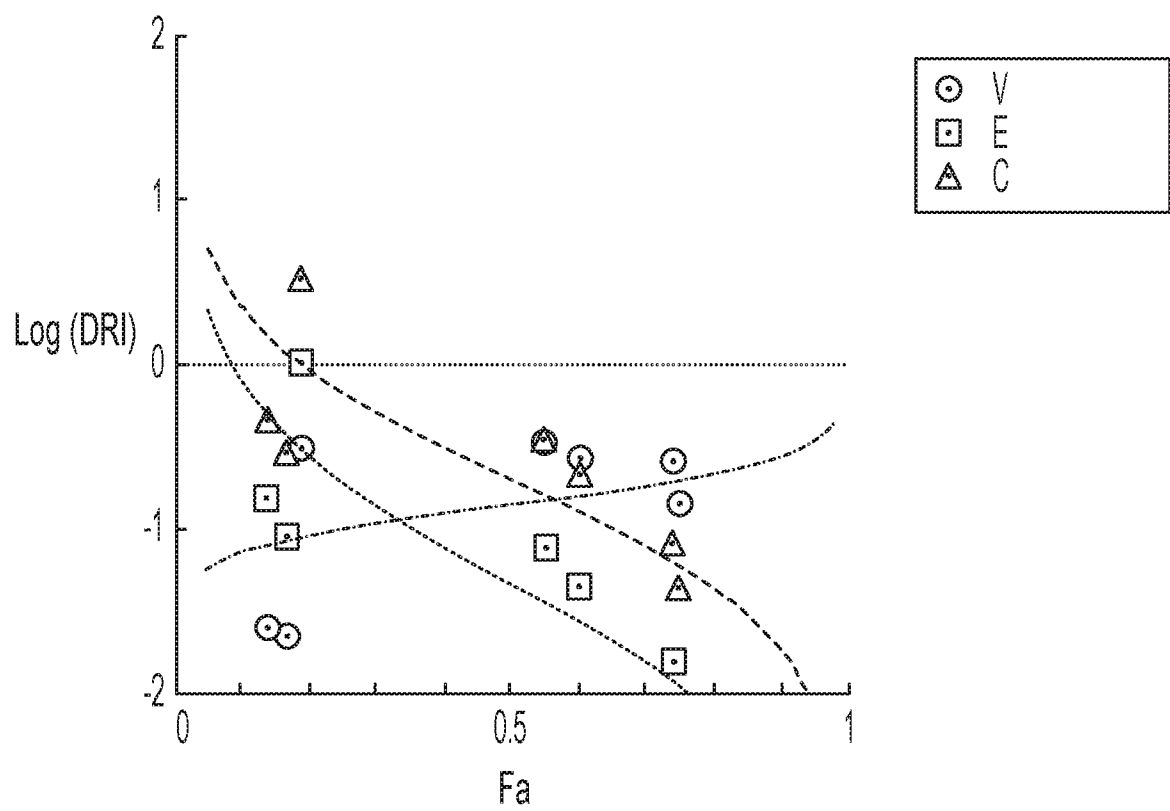


FIG. 4C

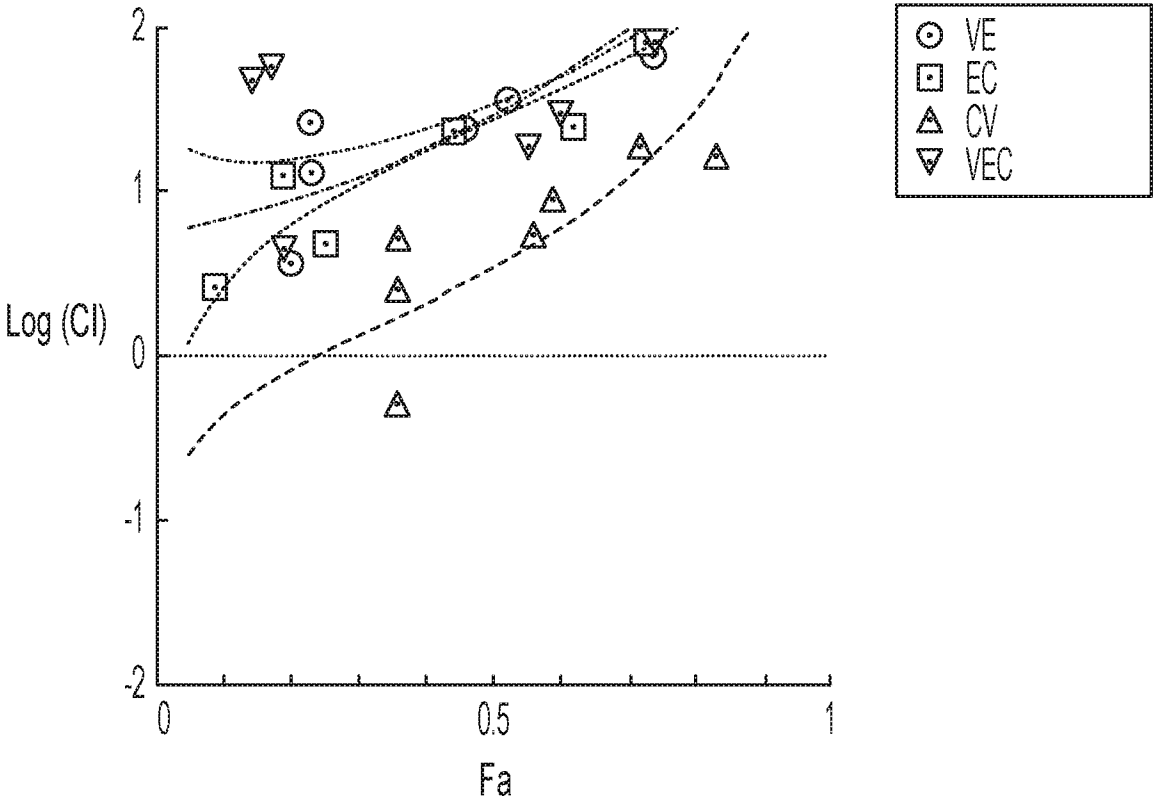
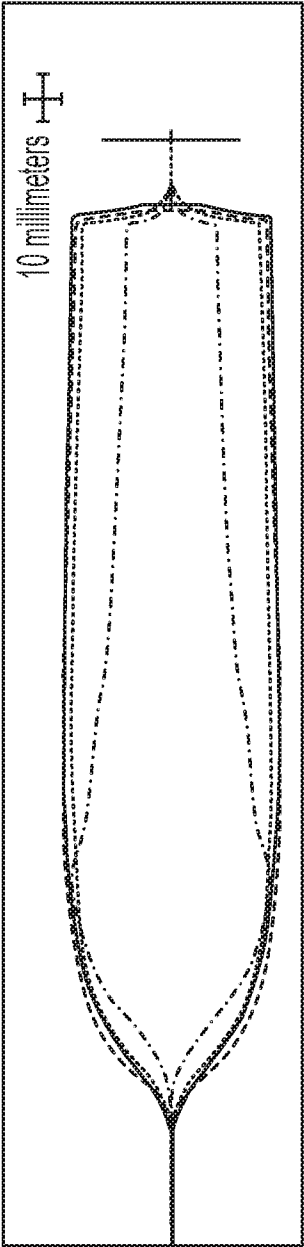


FIG. 4D

TEG® Analysis Results

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Tracings from top to bottom:

Seq	Channel	Patient	ST	Sample description	Operator	Analyzer SN
1	1	073119_CH -- 073119_CH	--	Saline Control_#1	Temporary Operator	0811-2428-mbb
2	2	073119_CH -- 073119_CH	--	Carboplatin (1mM)_#1	Temporary Operator	0811-2428-mbb
3	3	073119_CH -- 073119_CH	--	Etoposide (1mM)_#1	Temporary Operator	0911-2428-mbb
4	4	073119_CH -- 073119_CH	--	Vincristine (1mM)_#1	Temporary Operator	0911-2428-mbb

FIG. 5A

Seq	Channel	R	K	Angle	MA	PMA	G	EPL	A	CI	LY30
		min	min	deg	mm		Kd/sc	%	mm		%
1	1	13.7	6.1	20.8	54.5		6.0	0.0	0.2		0.0
2	2	14.8	4.2	40.3	55.4		6.2	0.1	0.1		0.1
3	3	14.7	5.8	30.3	50.7		5.1	0.0	0.1		0.0
4	4	17.9	6.2	32.7	50.8		5.2	20.6	0.1		20.6
Mean		15.3	5.6	31.0	52.9		5.6	5.2	0.1		5.2
n		4	4	4	4		4	4	4		4
SD		1.8	0.9	8.0	2.5		0.6	10.3	0.0		10.3
Min		13.7	4.2	20.8	50.7		5.1	0.0	0.1		0.0
Max		17.9	6.2	40.3	55.4		6.2	20.6	0.2		20.6

FIG. 5A CONT.

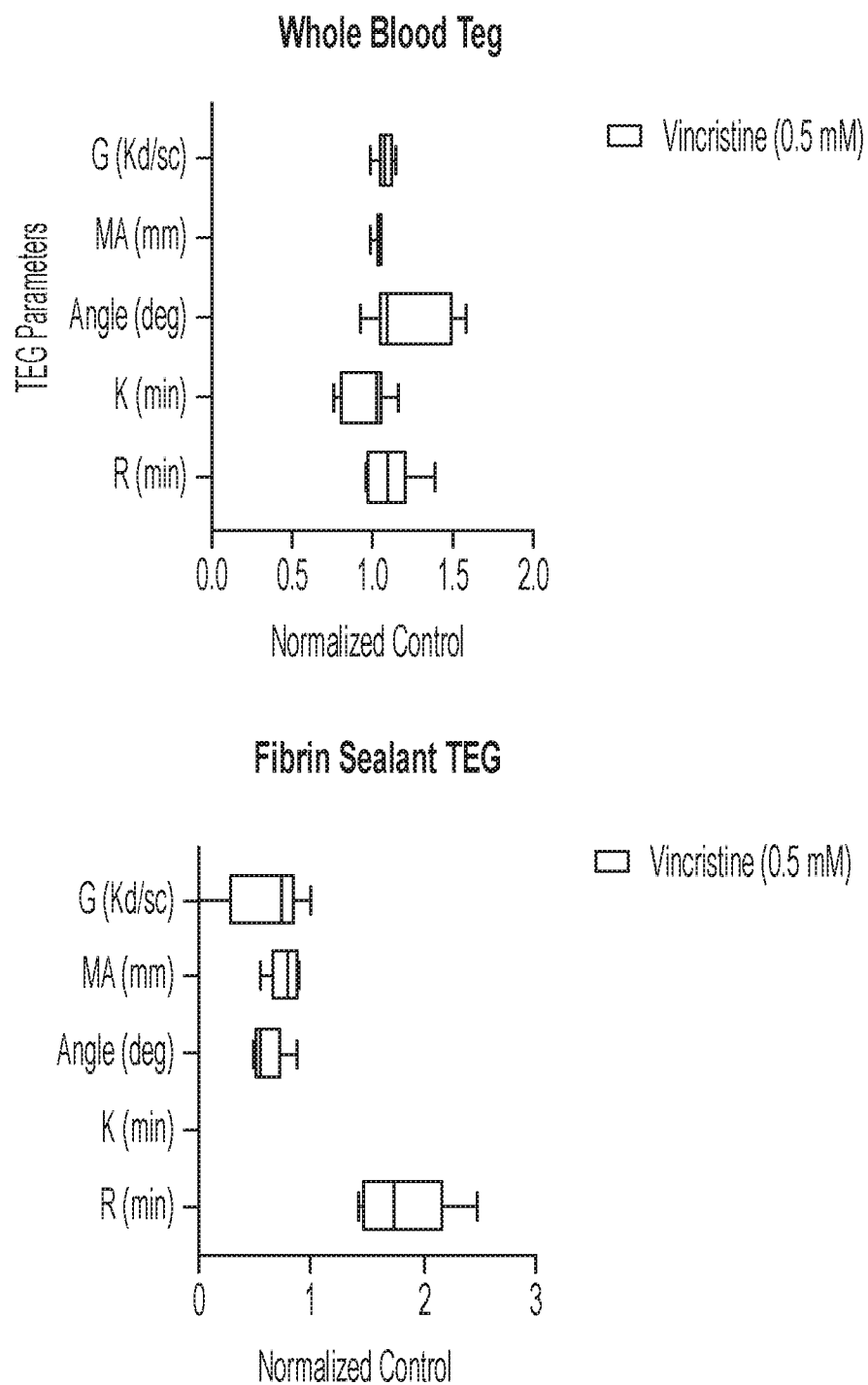
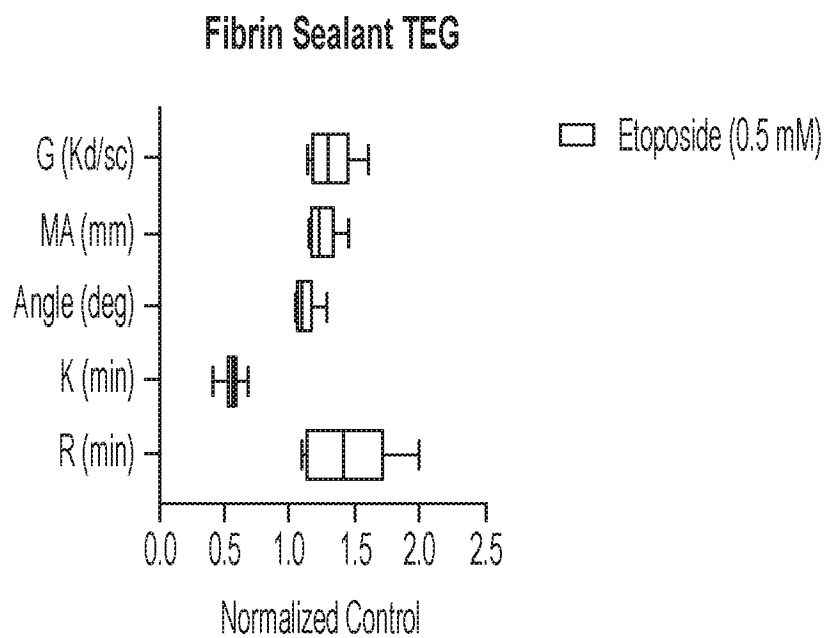
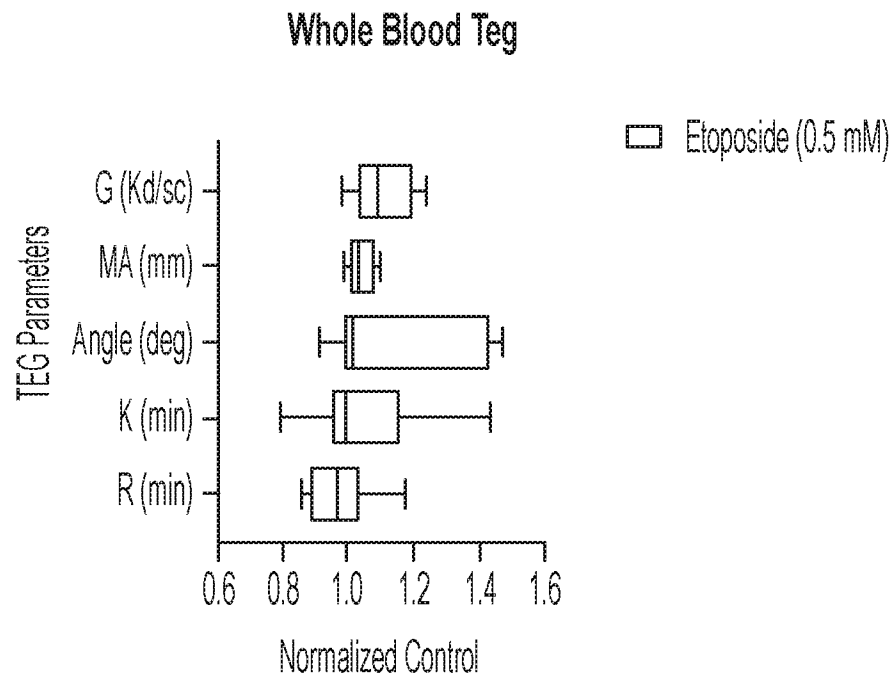
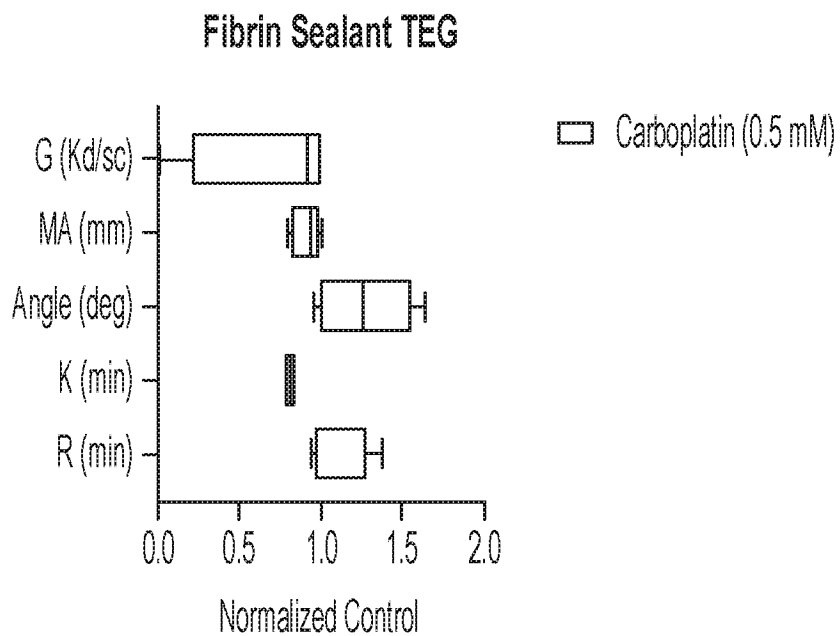
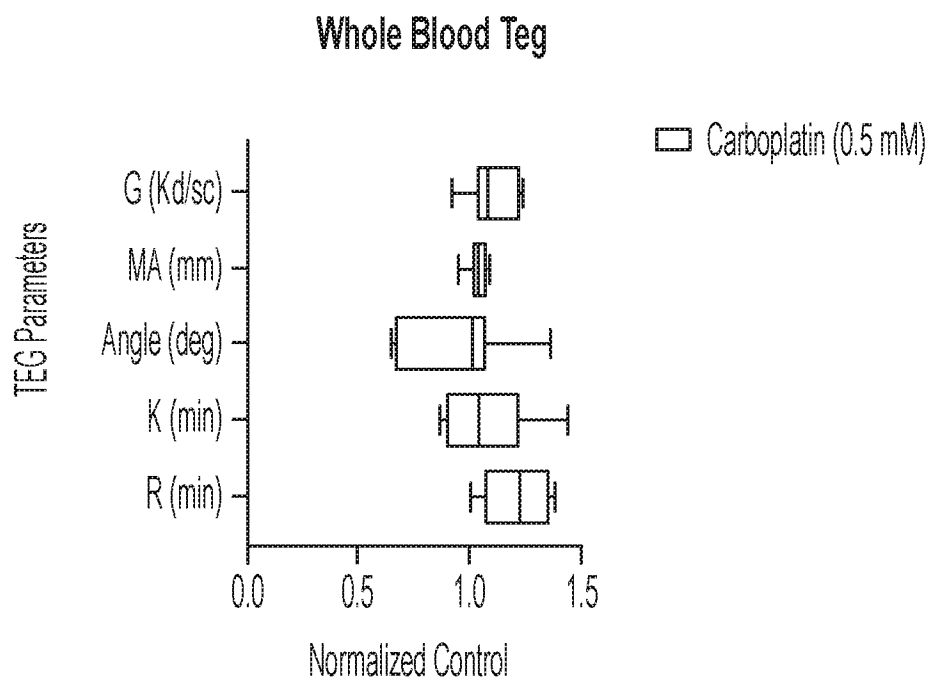


FIG. 5B

**FIG. 5B CONT.**

**FIG. 5B CONT.**

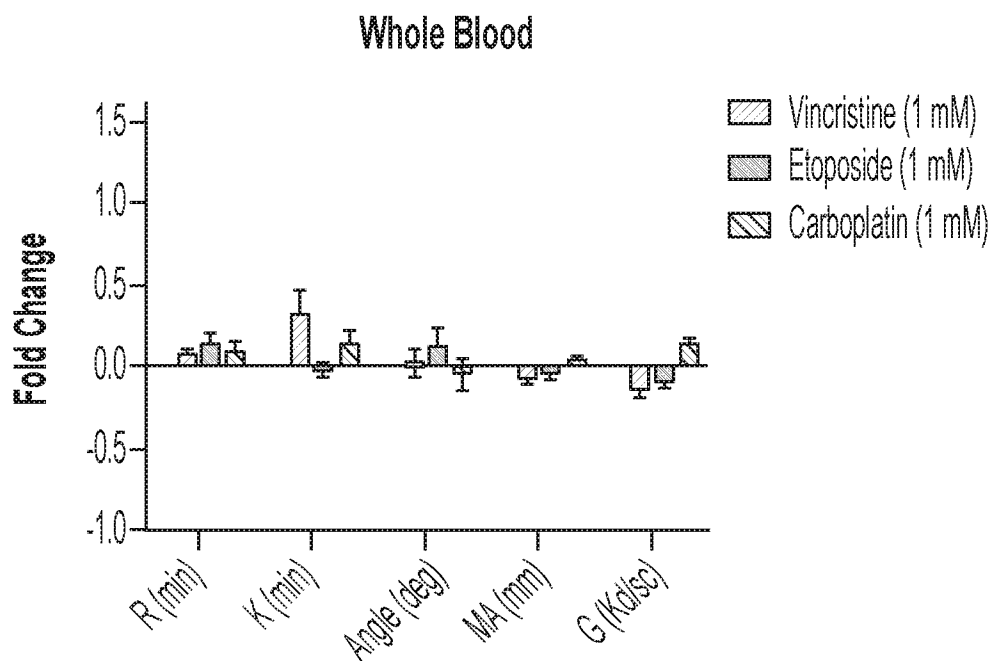


FIG. 6A

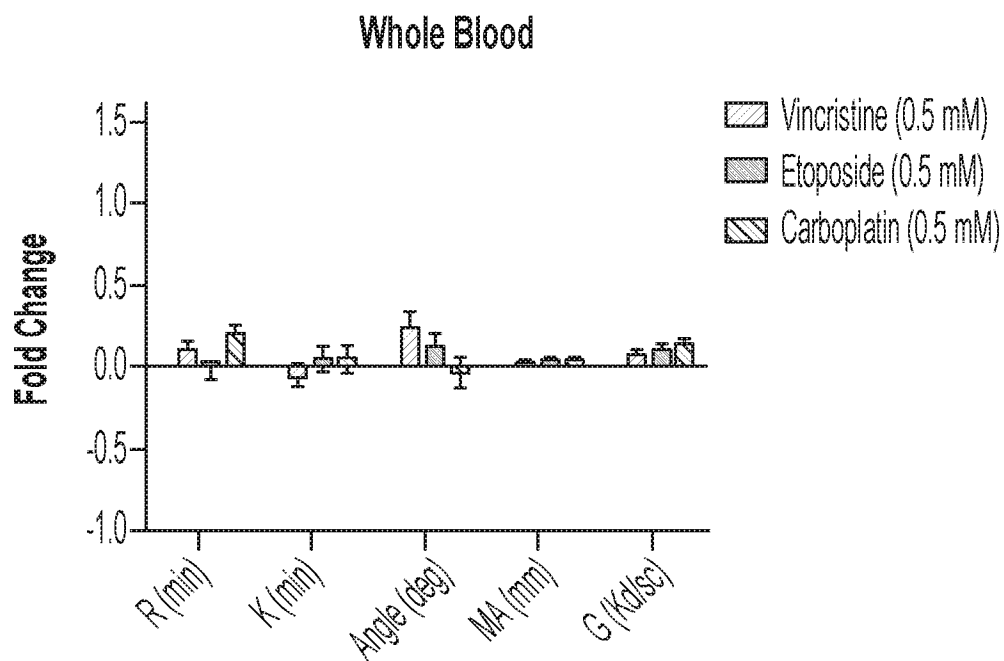


FIG. 6B

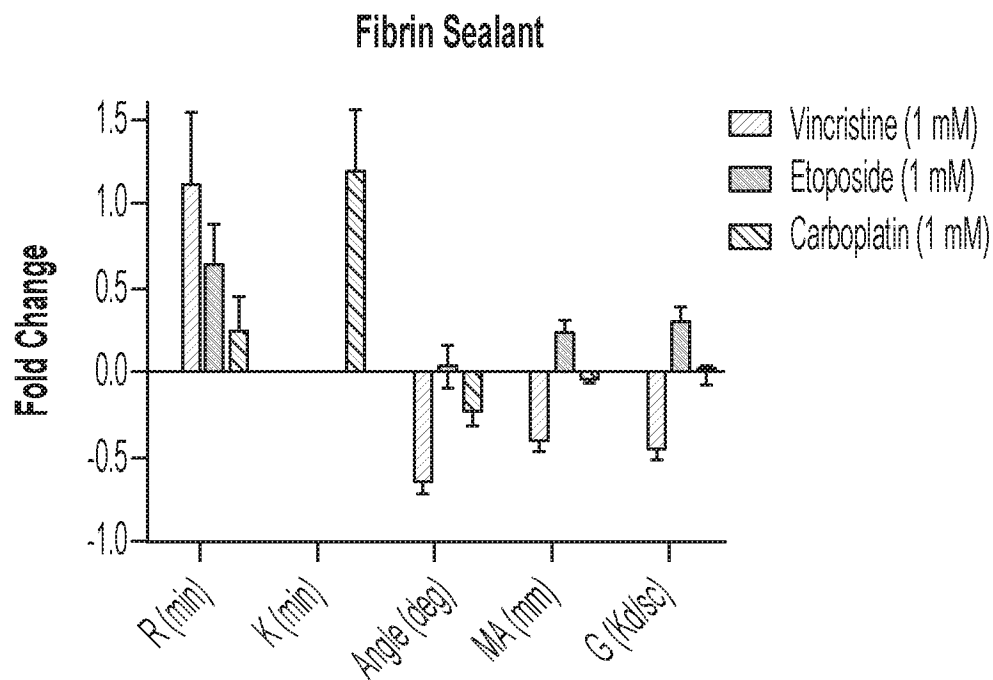


FIG. 6C

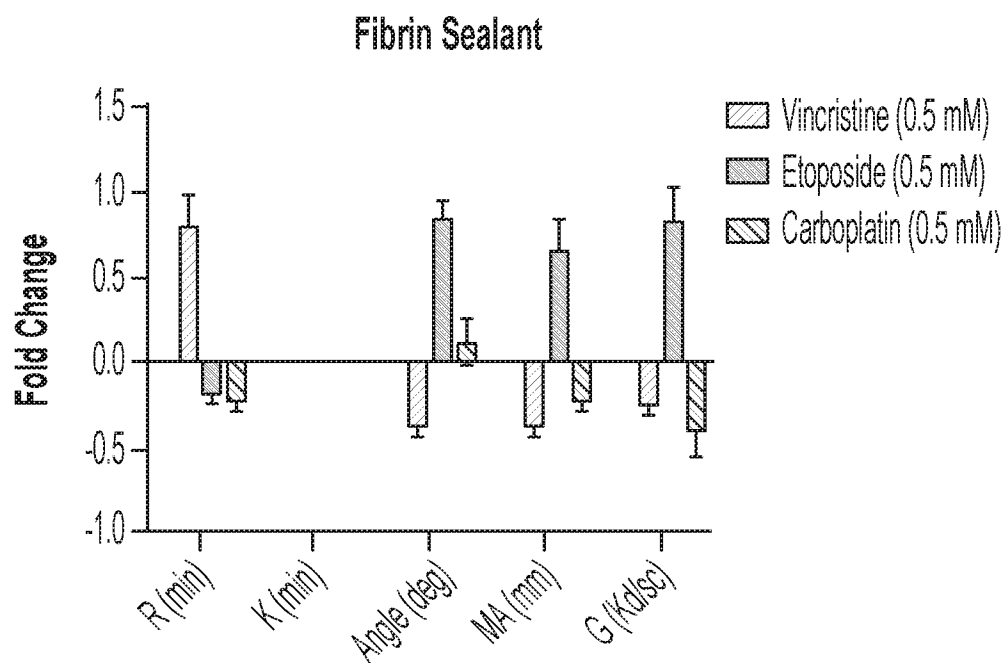


FIG. 6D

Whole Blood			
0.5 mM			
Vincristine	Etoposide	Carboplatin	
R (min)	11.5 ± 5.8	-1.5 ± 3.9	20.3 ± 5.3
K (min)	4.5 ± 5.8	5.6 ± 7.5	5.8 ± 7.8
Angle (deg)	24.3 ± 10.3	12.5 ± 8.7	-3.2 ± 9.2
MA (mm)	2.9 ± 0.7	4.3 ± 1.7	3.8 ± 1.8
G (Kd/sc)	8.2 ± 2.3	10.9 ± 3.9	10.6 ± 4.4

Fibrin Sealant			
0.5 mM			
Vincristine	Etoposide	Carboplatin	
R (min)	78.5 ± 19.2	-19.2 ± 5.9	-24.2 ± 4.7
K (min)	0 ± 0	0 ± 0	0 ± 0
Angle (deg)	-37.9 ± 6.7	83.2 ± 11.8	12.2 ± 14.3
MA (mm)	-21.4 ± 6.3	64.8 ± 19.1	-24.8 ± 4.3
G (Kd/sc)	-25.7 ± 5.6	82 ± 19.8	-40.5 ± 15.2

Whole Blood			
1 mM			
Vincristine	Etoposide	Carboplatin	
R (min)	7.5 ± 3.8	14.2 ± 5.3	9.4 ± 6.9
K (min)	32.8 ± 13.7	-1.7 ± 3.3	15.6 ± 5.4
Angle (deg)	2.2 ± 7.8	13.4 ± 9.6	-4.2 ± 9.3
MA (mm)	-7.4 ± 2.8	-4.1 ± 2.8	5.2 ± 1.1
G (Kd/sc)	-13.6 ± 6	-7.5 ± 5.6	14.4 ± 3.2

Fibrin Sealant			
1 mM			
Vincristine	Etoposide	Carboplatin	
R (min)	113 ± 42.5	64 ± 23.4	24.2 ± 21.1
K (min)	0 ± 0	0 ± 0	120.9 ± 35.8
Angle (deg)	-64.6 ± 6	4 ± 12.5	-22.7 ± 7.7
MA (mm)	-39.8 ± 7	23.7 ± 6.9	-3.4 ± 2.6
G (Kd/sc)	-43.5 ± 8.2	31 ± 8.1	-1.2 ± 5.4

FIG. 6E

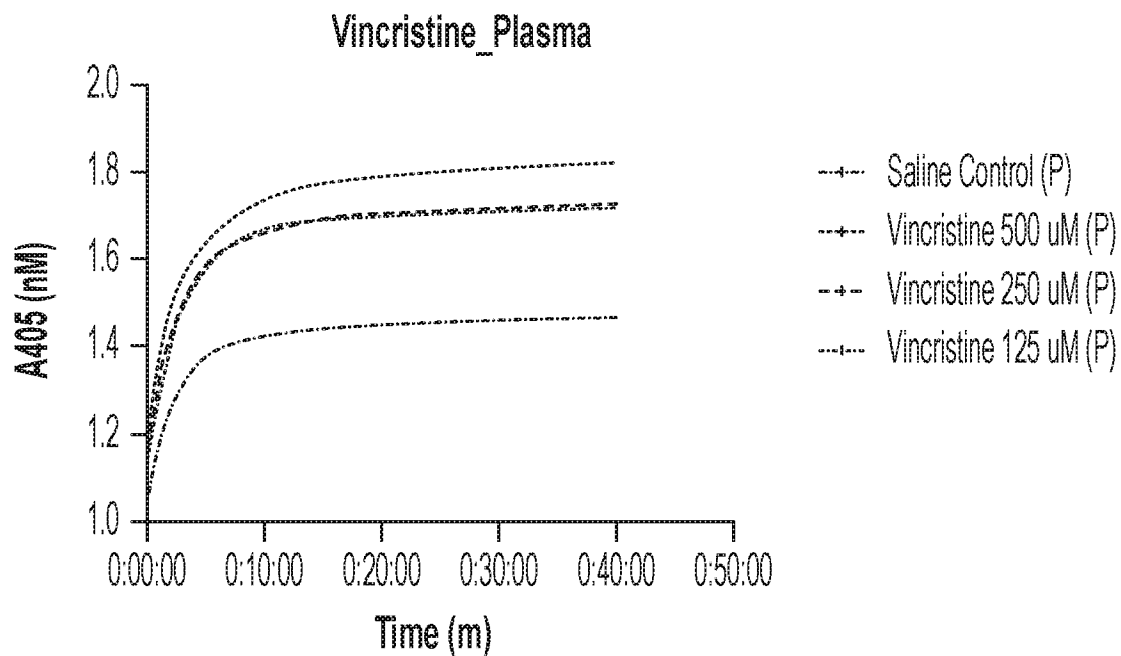


FIG. 7A

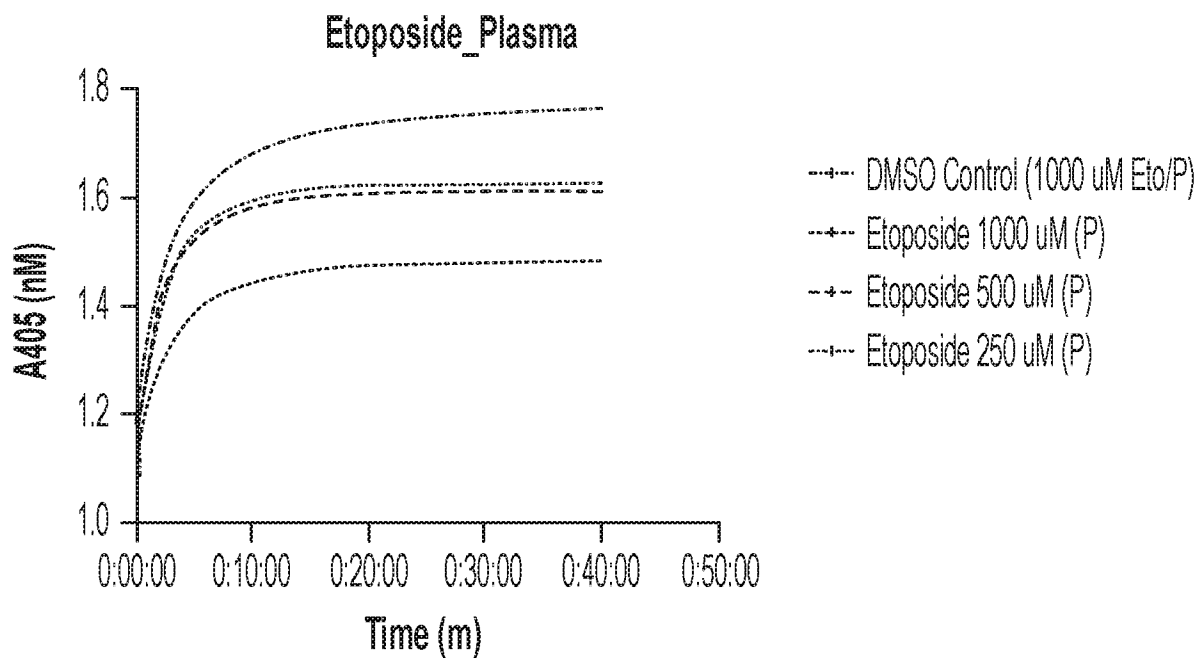
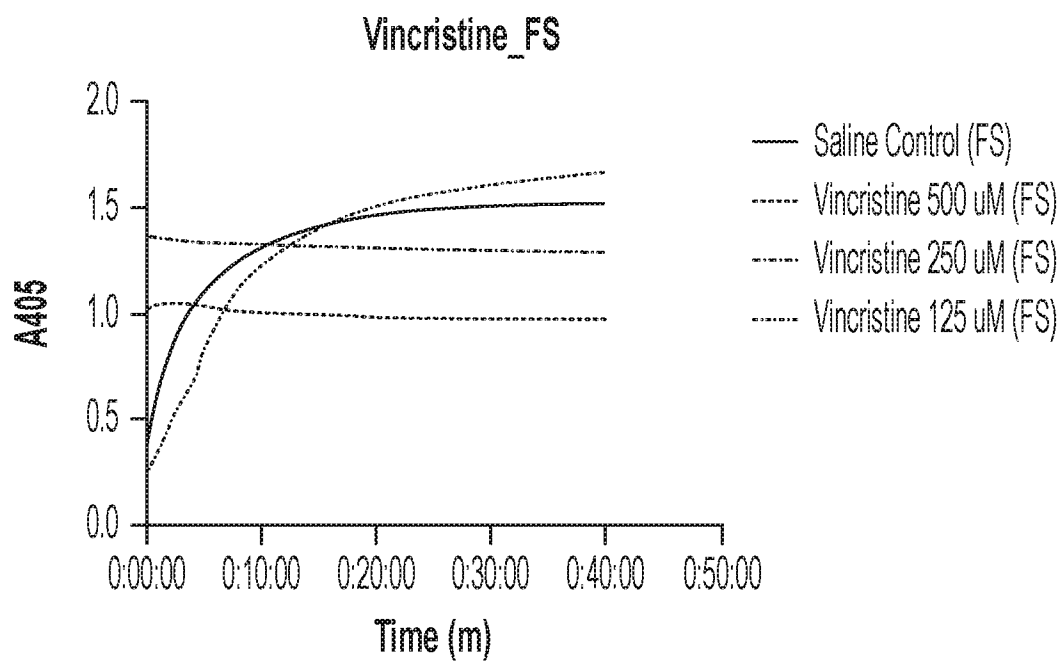
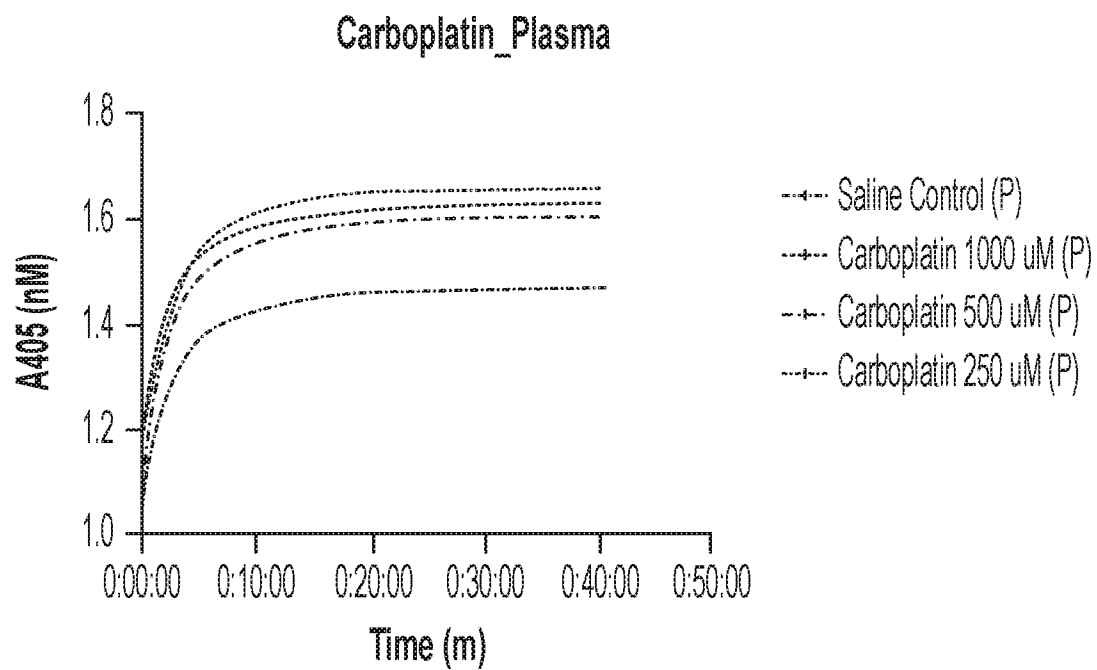


FIG. 7B



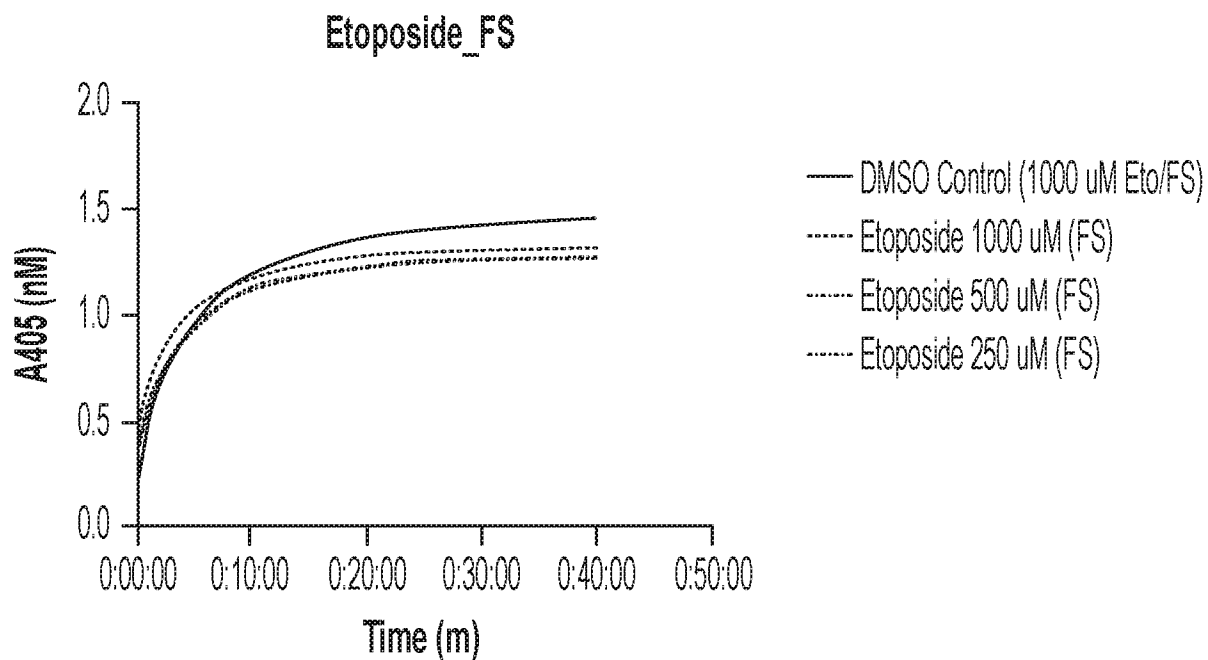


FIG. 7E

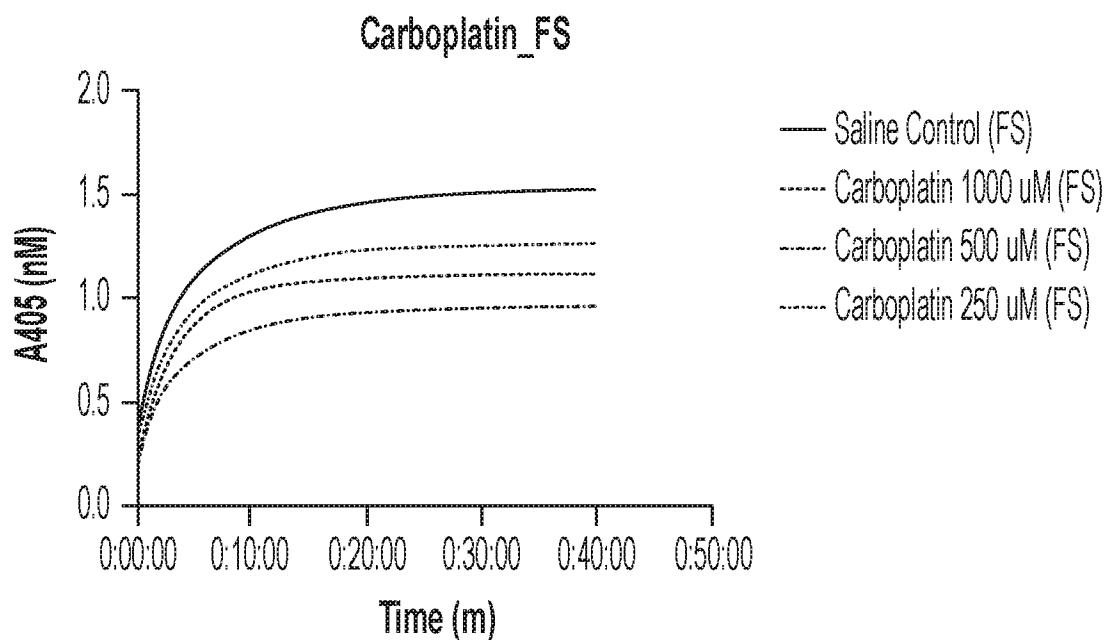


FIG. 7F

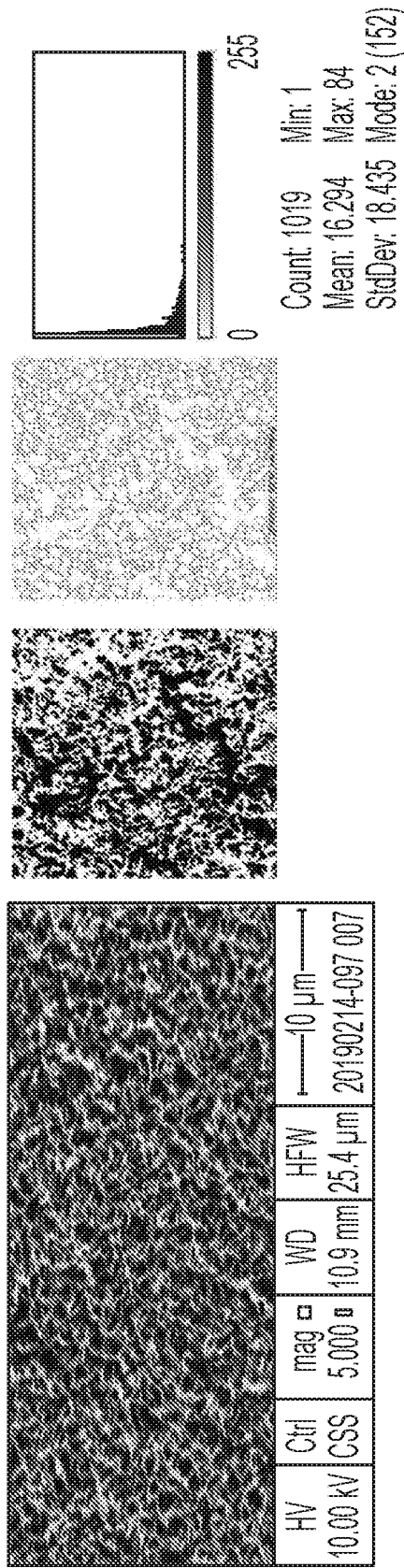


FIG. 8

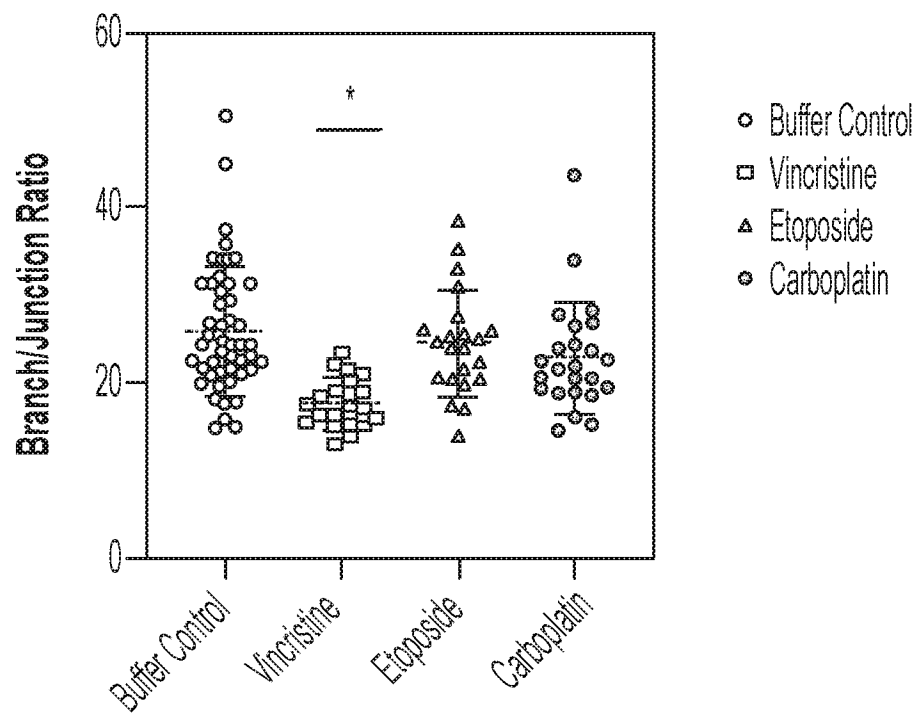


FIG. 9

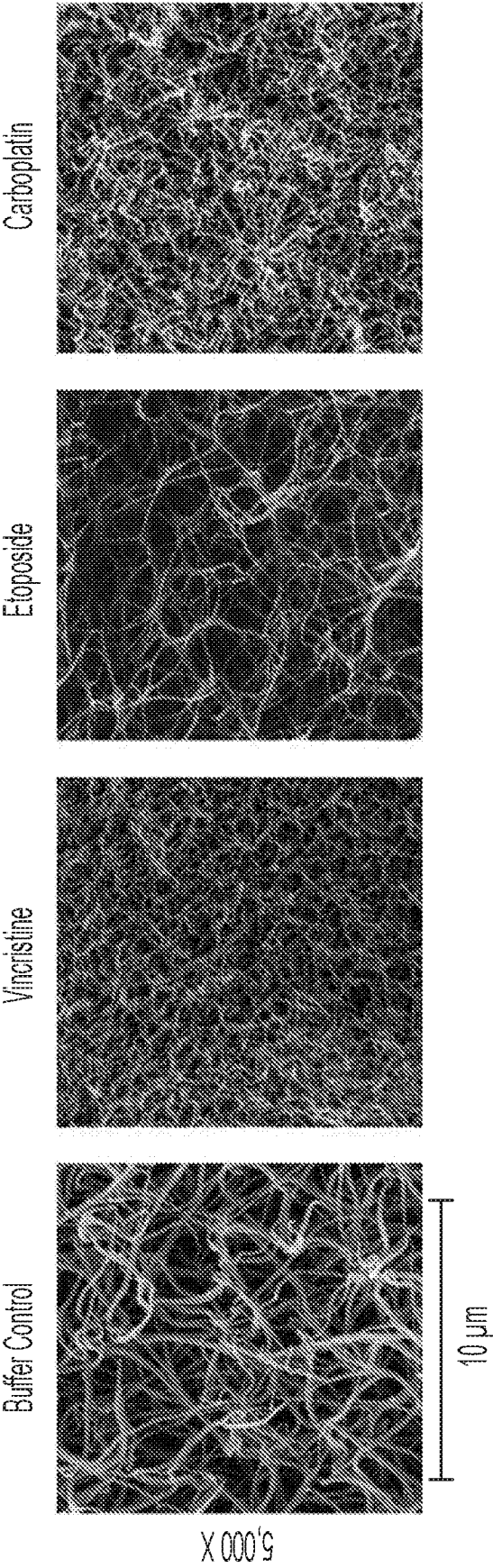


FIG. 10

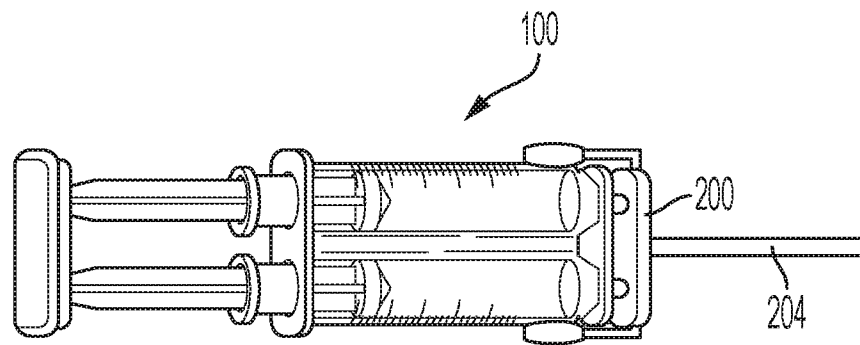


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019915

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K38/36 A61K31/00	A61K38/48 A61K31/475
	A61P35/00 A61M5/178	A61K31/7048 A61J1/20
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61P A61J A61M		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN QUILL ET AL: "Subconjunctival Carboplatin in Fibrin Sealant in the Treatment of Transgenic Murine Retinoblastoma", OPTHALMOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 112, no. 6, 31 May 2005 (2005-05-31), pages 1151-1158, XP005825984, ISSN: 0161-6420, DOI: 10.1016/J.OPHTHA.2004.11.060	1-41
Y	abstract page 1151, left-hand column, paragraph 1 - page 1157, left-hand column, paragraph 4 ----- -/-	1-41
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 20 May 2020		Date of mailing of the international search report 04/06/2020
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Camilleri, Alain

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019915

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAIROOZ P. MANJANDAVIDA ET AL: "Management and Outcome of Retinoblastoma with Vitreous Seeds", OPHTHALMOLOGY, vol. 121, no. 2, 25 October 2013 (2013-10-25), pages 517-524, XP055696614, AMSTERDAM, NL ISSN: 0161-6420, DOI: 10.1016/j.optho.2013.09.011	1-41
Y	abstract page 517, left-hand column, paragraph 1 - page 523, right-hand column, paragraph 2 -----	1-41
X	SCHMACK ET AL: "Ischemic Necrosis and Atrophy of the Optic Nerve After Periocular Carboplatin Injection for Intraocular Retinoblastoma", AMERICAN JOURNAL OF OPHTHALMOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 142, no. 2, 1 August 2006 (2006-08-01), pages 310-315.e2, XP005577377, ISSN: 0002-9394, DOI: 10.1016/J.AJO.2006.02.044	1-41
Y	abstract page 310, right-hand column, paragraph 1 - page 315, left-hand column, paragraph 2 -----	1-41
X	RIPAL GAUDANA ET AL: "Ocular Drug Delivery", THE AAPS JOURNAL, vol. 12, no. 3, 1 September 2010 (2010-09-01), pages 348-360, XP055060472, ISSN: 1550-7416, DOI: 10.1208/s12248-010-9183-3	1-41
Y	abstract page 348, left-hand column, paragraph 1 - page 357, left-hand column, paragraph 2 -----	1-41
X	NANCY E. MARTIN ET AL: "Fibrin Sealant for Retinoblastoma: Where Are We?", JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS., vol. 24, no. 5, 1 October 2008 (2008-10-01), pages 433-438, XP055697140, US ISSN: 1080-7683, DOI: 10.1089/jop.2007.0110	1-41
Y	abstract page 433, left-hand column, paragraph 1 - page 437, right-hand column, paragraph 3 -----	1-41
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/019915

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YACoub A. YOUSEF ET AL: "No ocular motility complications after subtenon topotecan with fibrin sealant for retinoblastoma", CANADIAN JOURNAL OF OPHTHALMOLOGY., vol. 48, no. 6, 1 December 2013 (2013-12-01), pages 524-528, XP055696616, CA ISSN: 0008-4182, DOI: 10.1016/j.jcjo.2013.05.018 abstract page 524, left-hand column, paragraph 1 - page 528, left-hand column, paragraph 2 -----	1-41
X	US 2012/195953 A1 (HOANG KIEU [US]) 2 August 2012 (2012-08-02)	17
Y	paragraph [0010] -----	1-41

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/019915

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012195953	A1	02-08-2012	NONE
