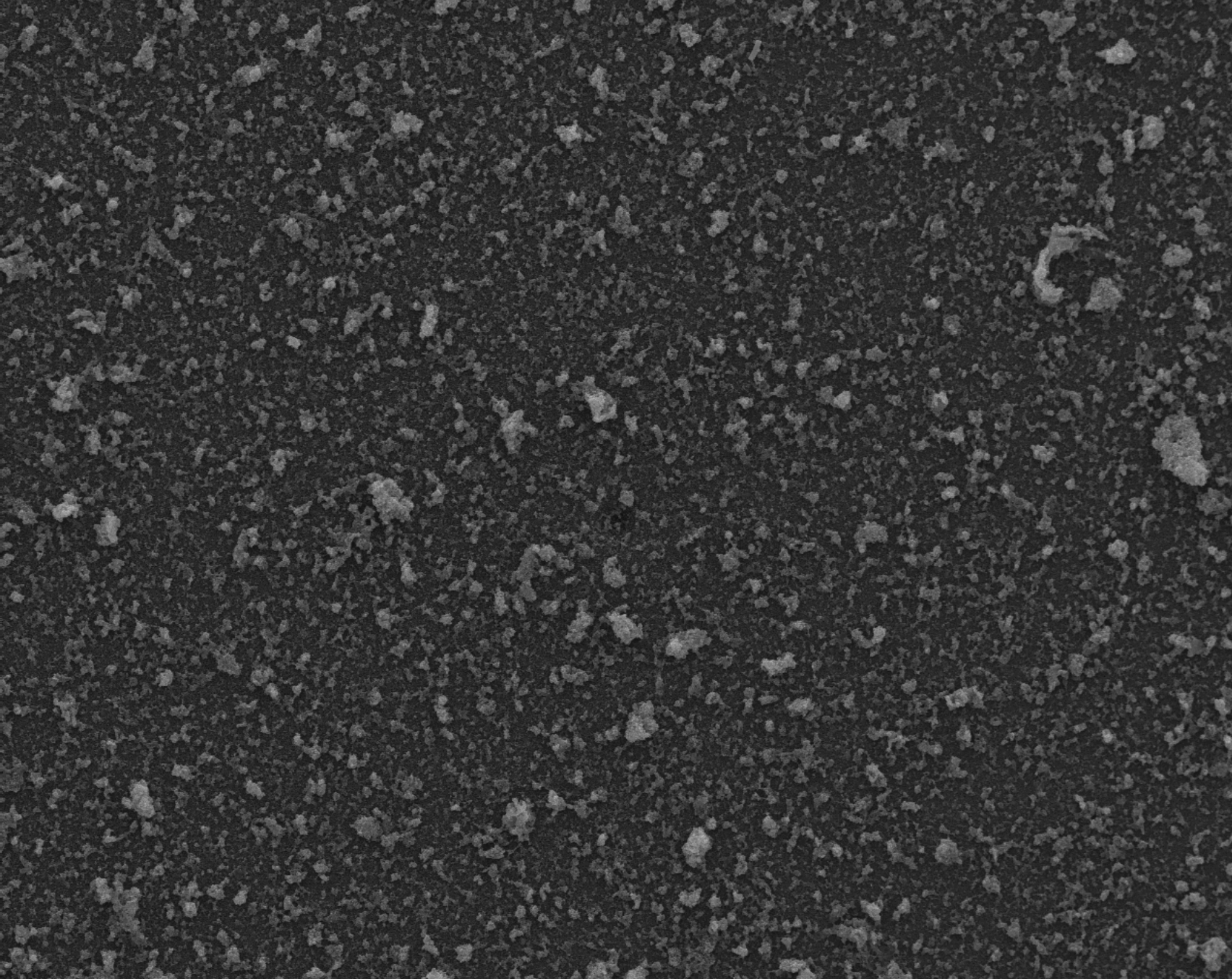


Isolate from spruce needle homogenate 1

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. *Cancers* 2022, 14, 3700. <https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

The samples were fixed with OsO₄ as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. *Int J Mol Sci.* 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO₄ for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).

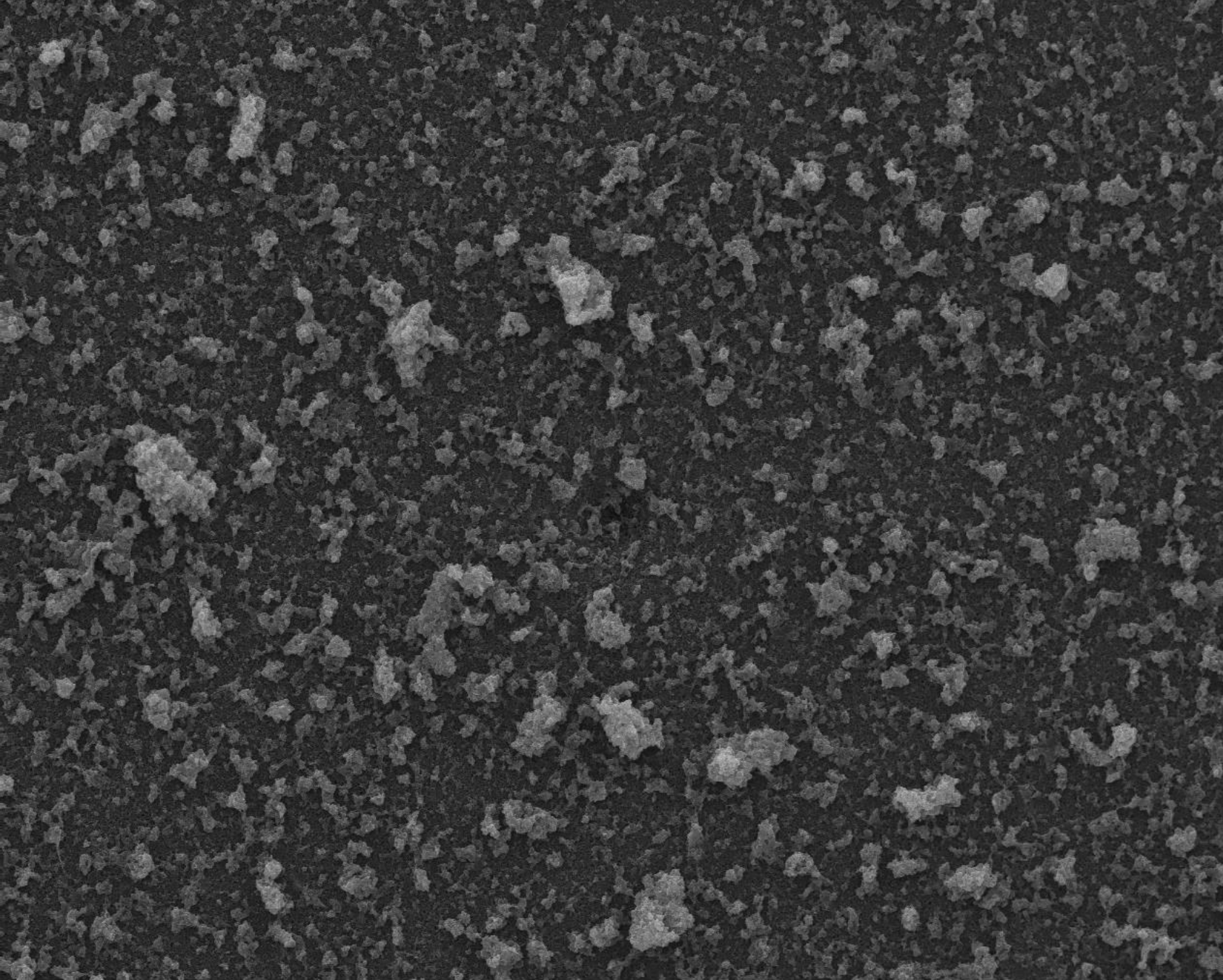


Isolate from spruce needle homogenate 2

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevnar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. *Cancers* 2022, 14, 3700. <https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

The samples were fixed with OsO₄ as adapted from (Božič, D.; Hočevnar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. *Int J Mol Sci.* 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO₄ for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).

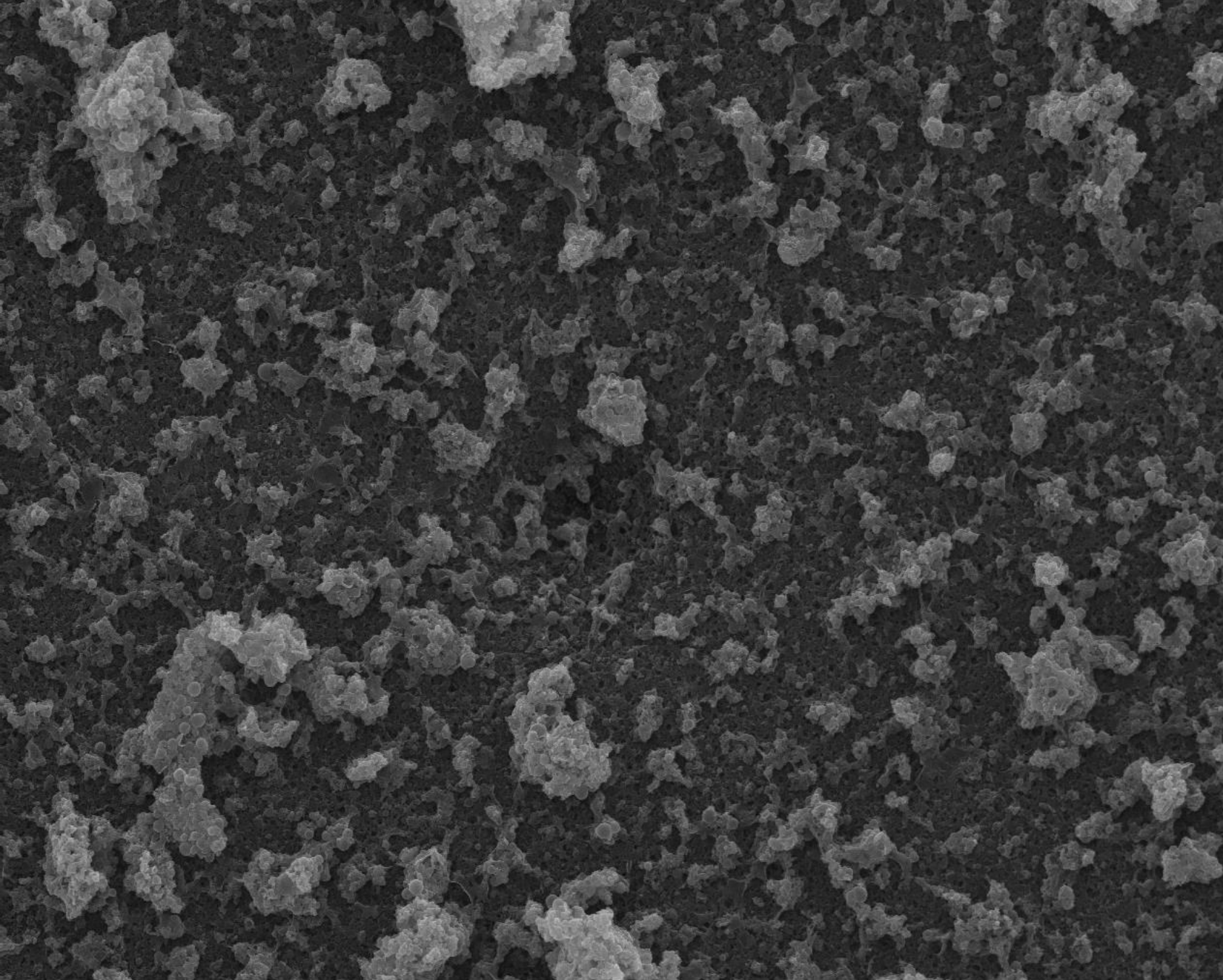


Isolate from spruce needle homogenate 3

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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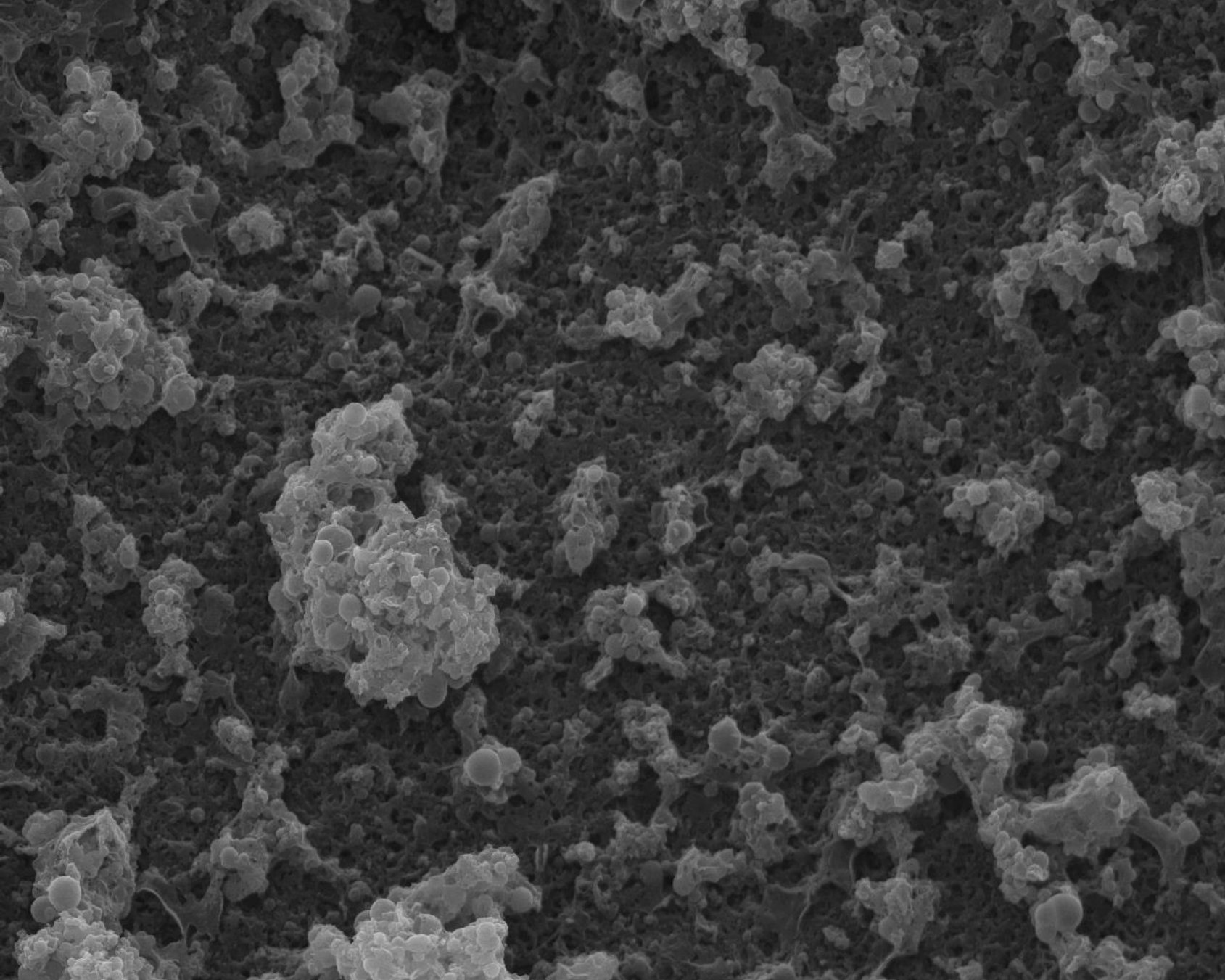
Isolate from spruce needle homogenate 4

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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<https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

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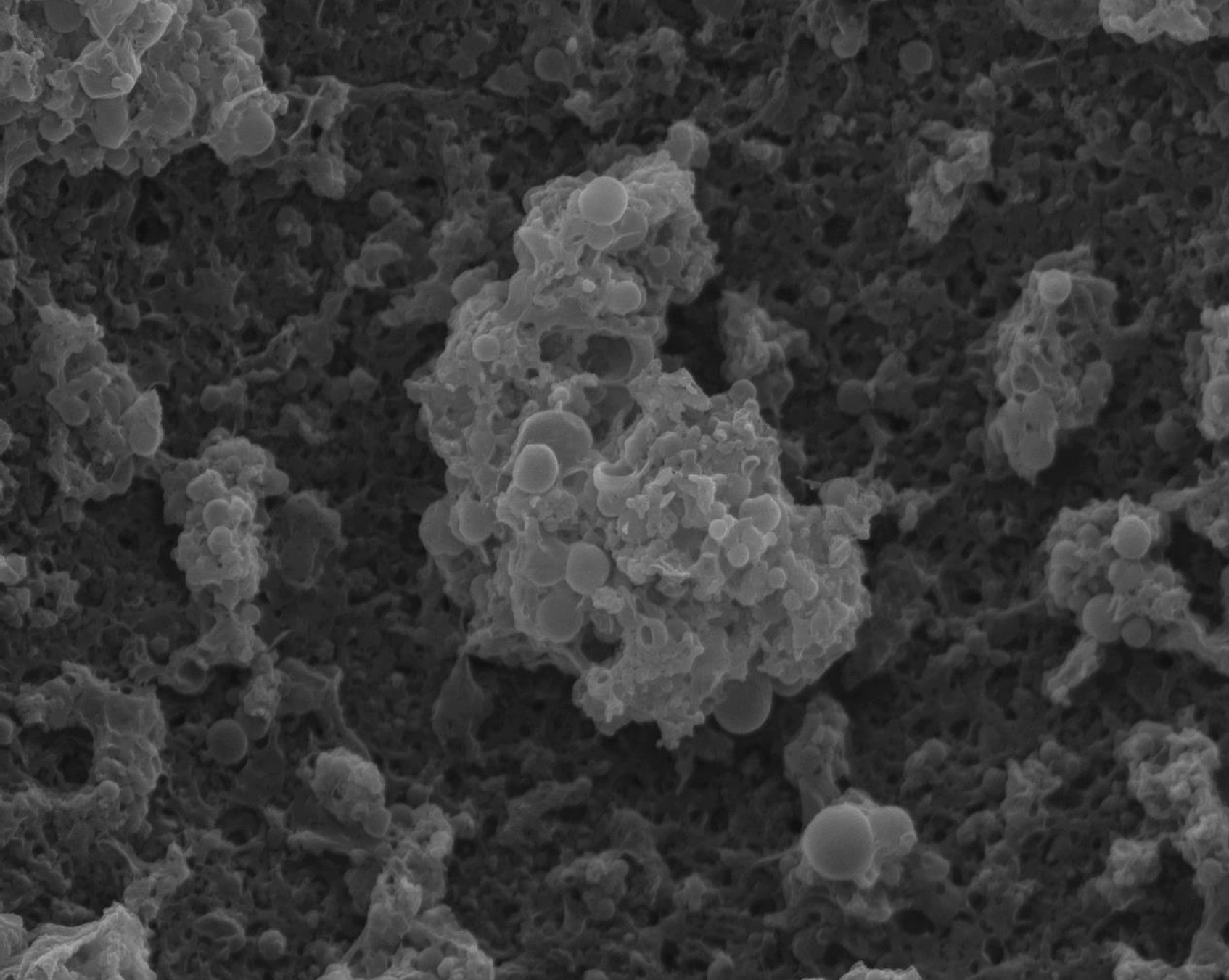
Isolate from spruce needle homogenate 5

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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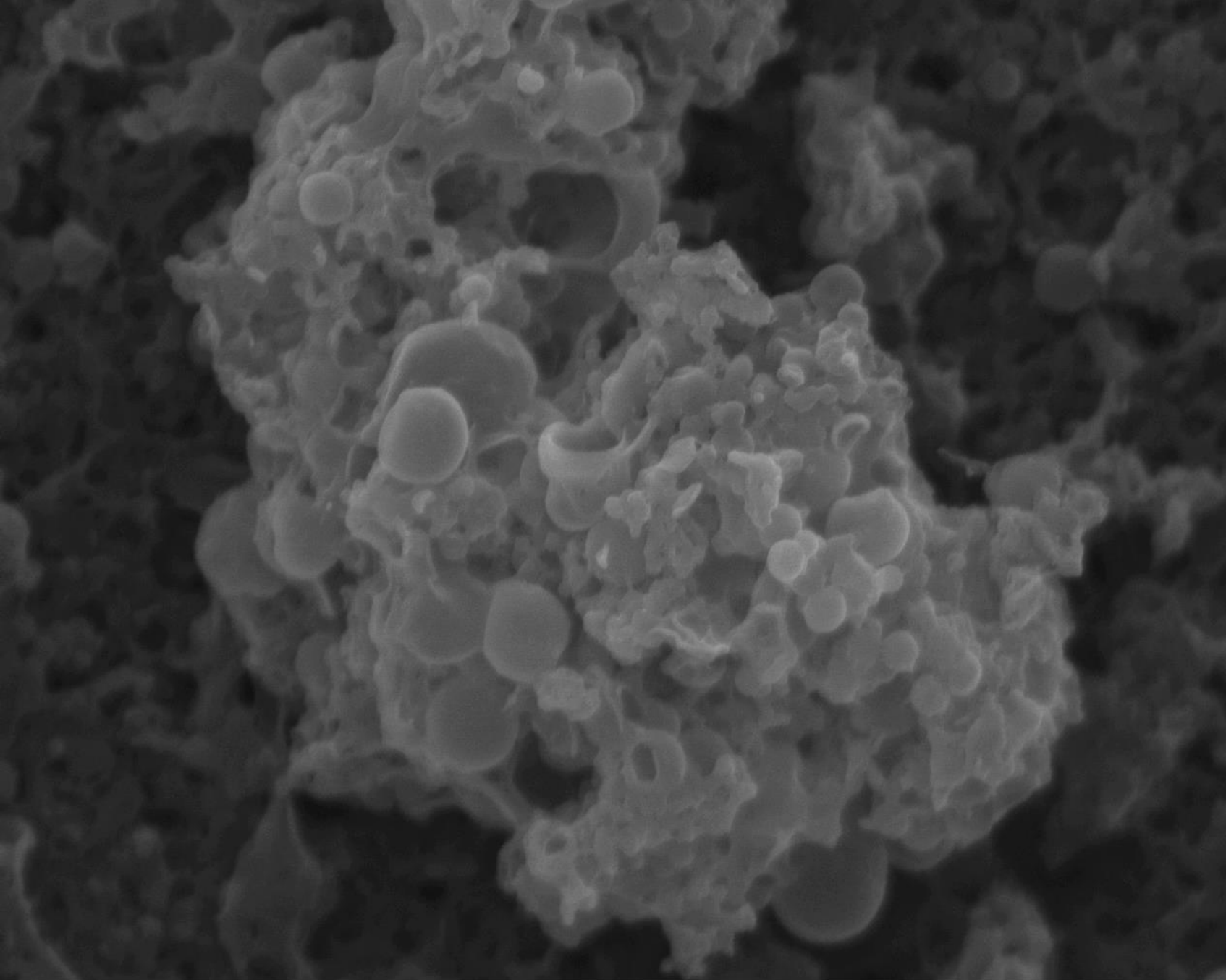
Isolate from spruce needle homogenate 6

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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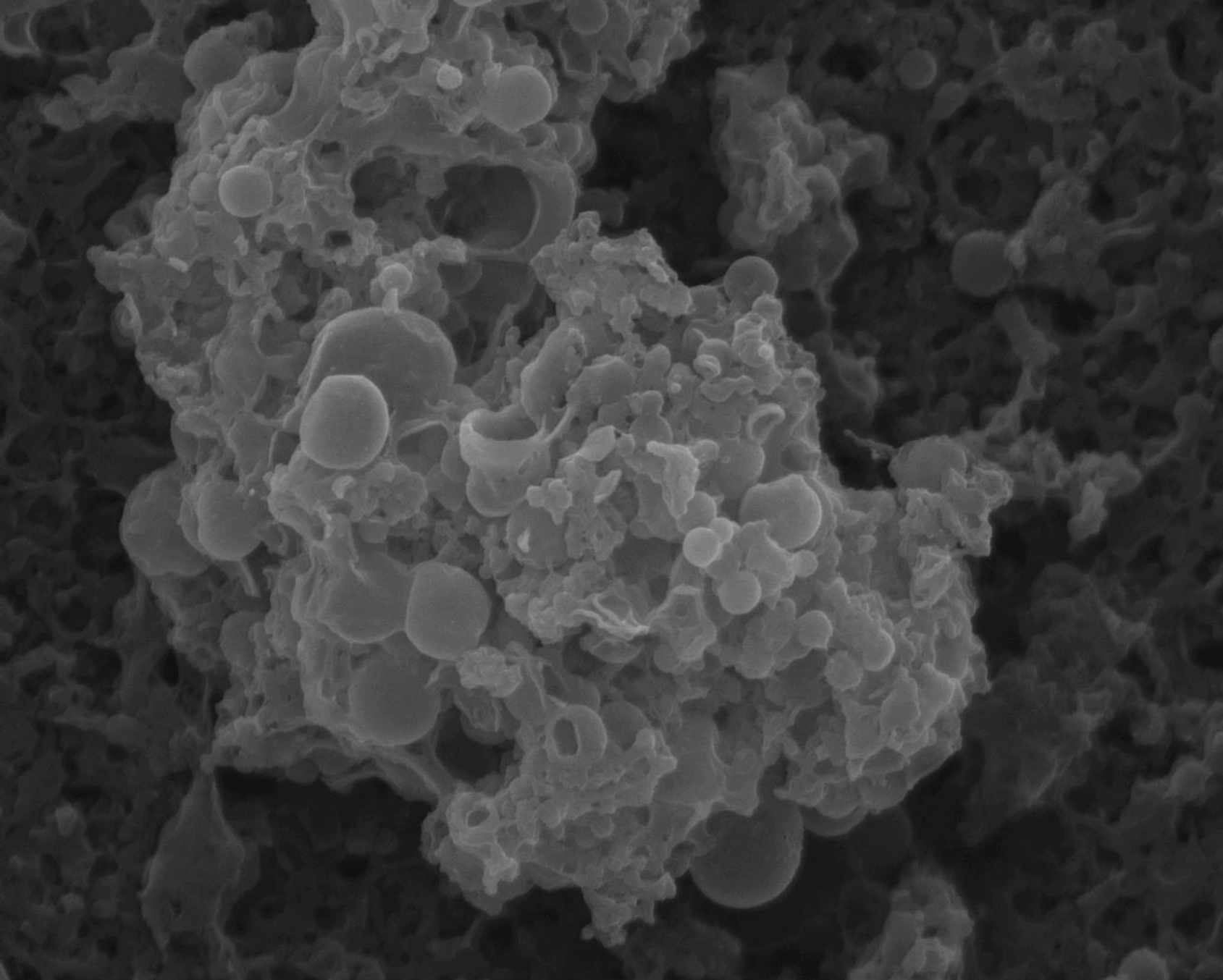
Isolate from spruce needle homogenate 7

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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<https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

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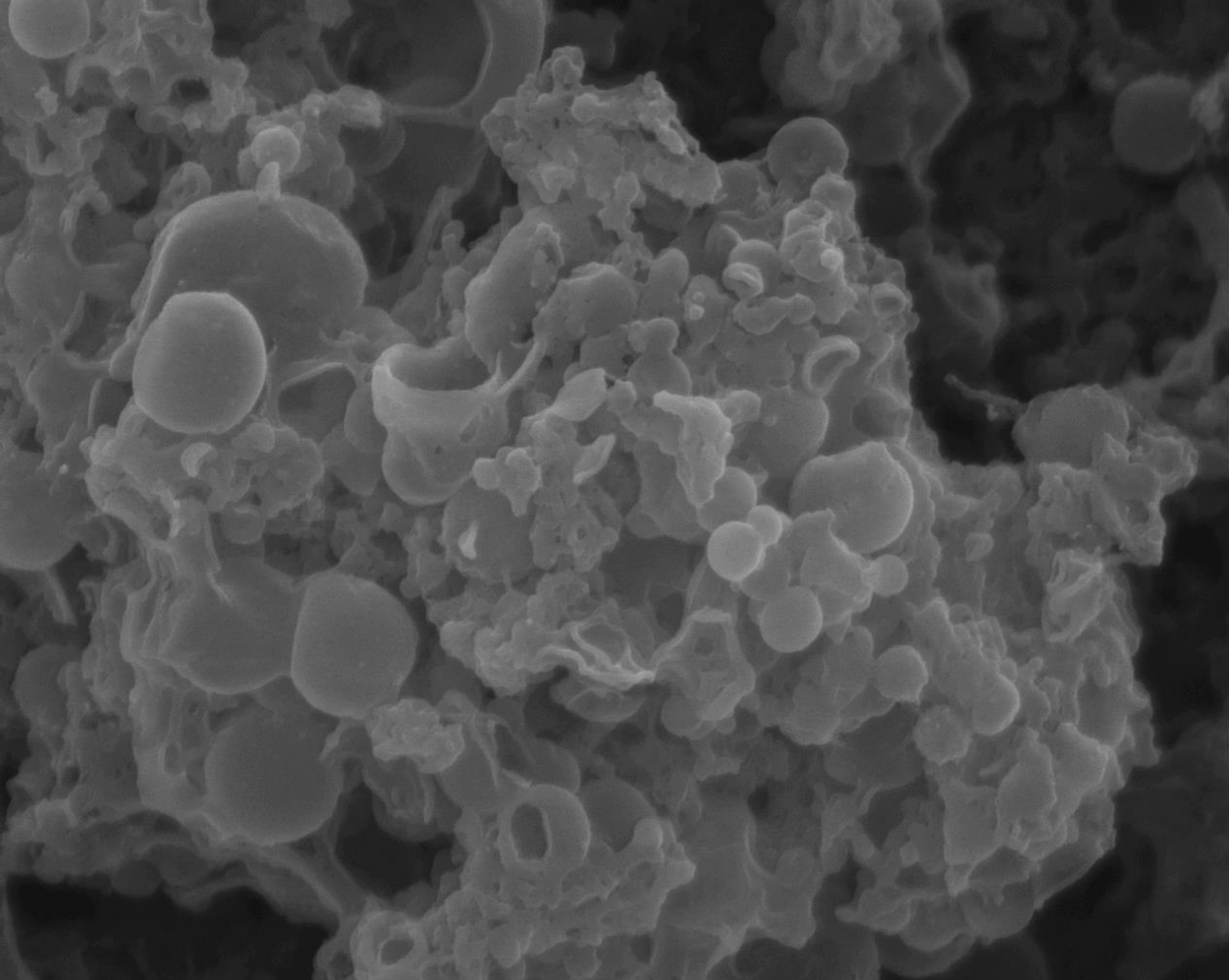
Isolate from spruce needle homogenate 8

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 μL of the pellet.

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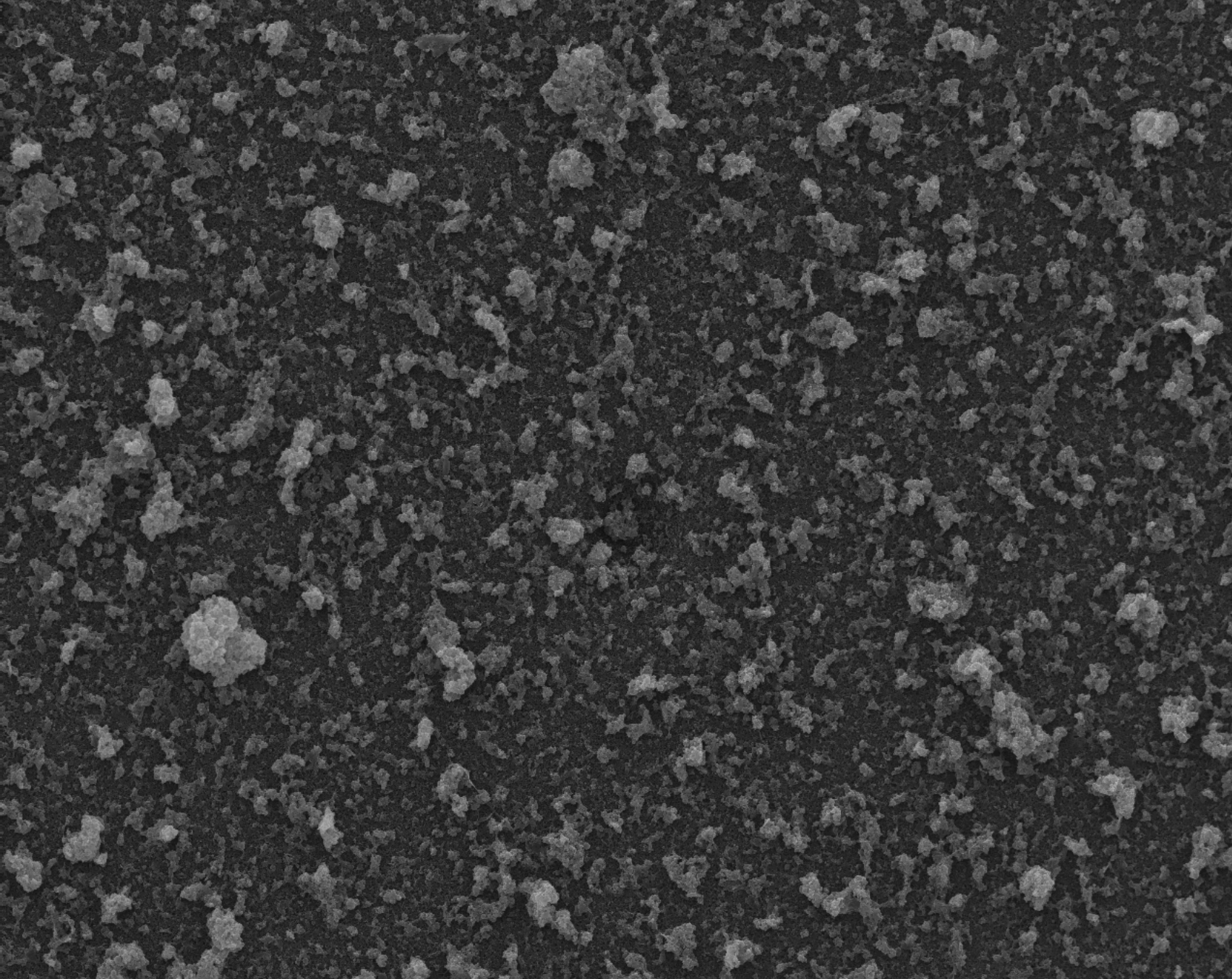
Isolate from spruce needle homogenate 9

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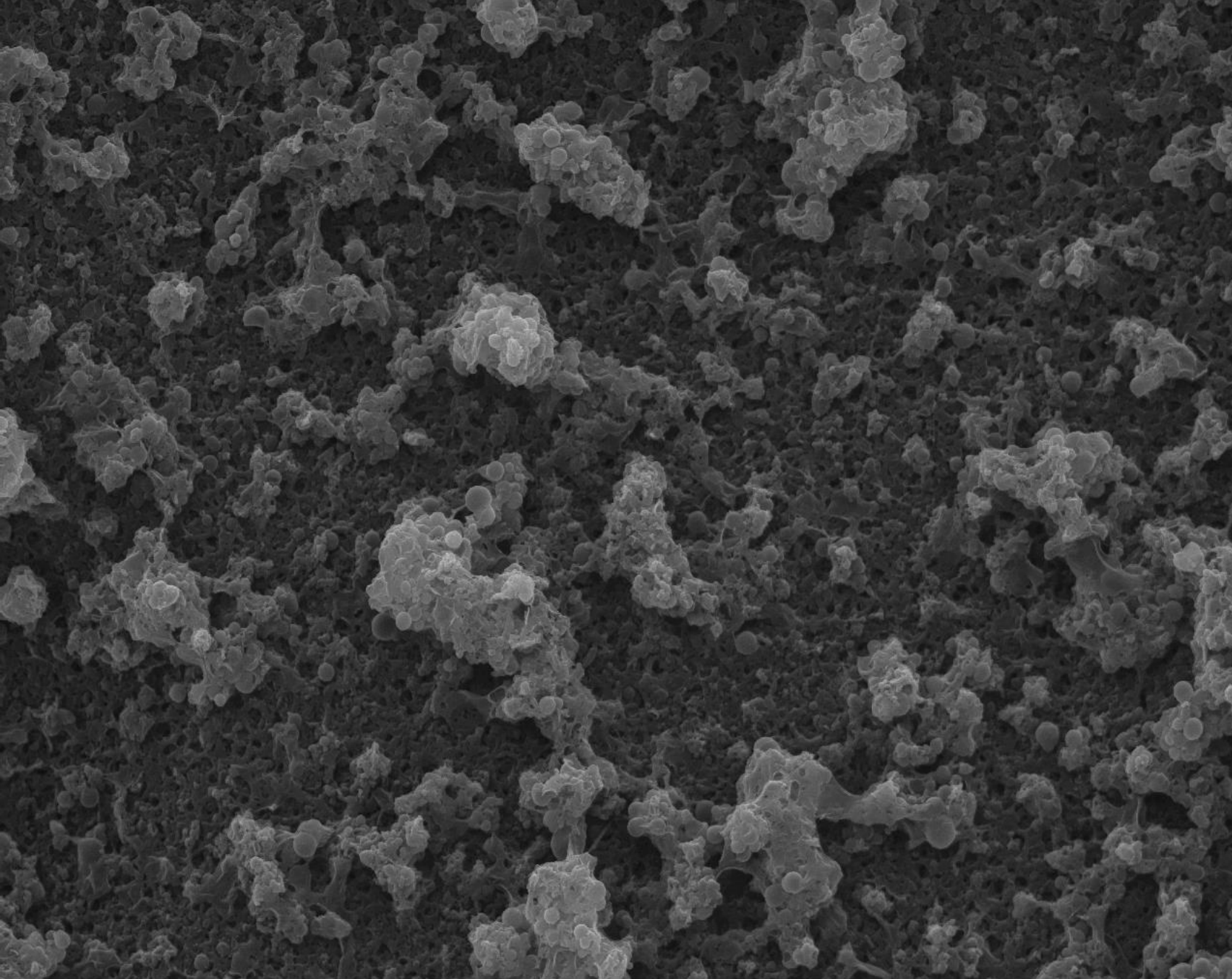
Isolate from spruce needle homogenate 10

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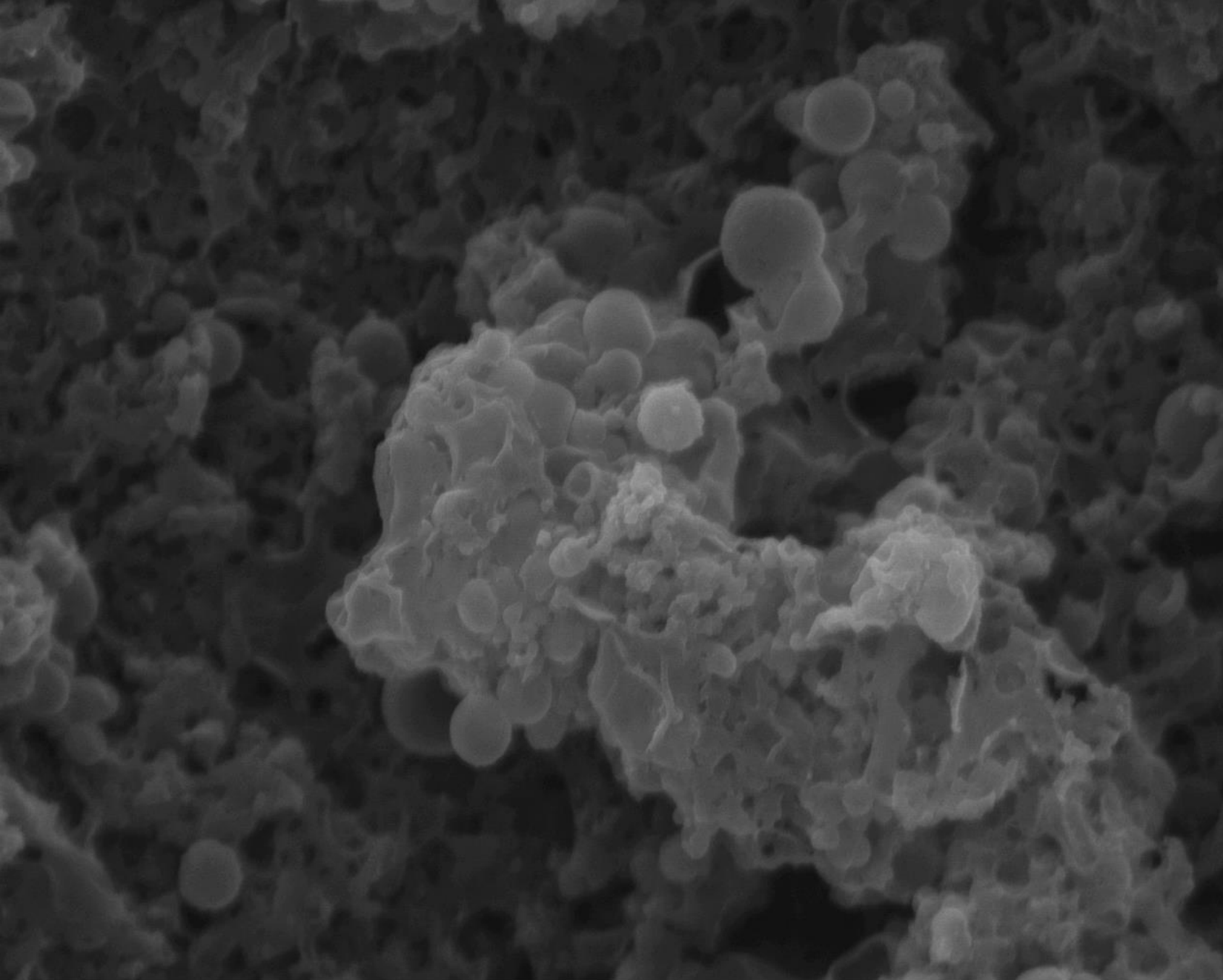
Isolate from spruce needle homogenate 11

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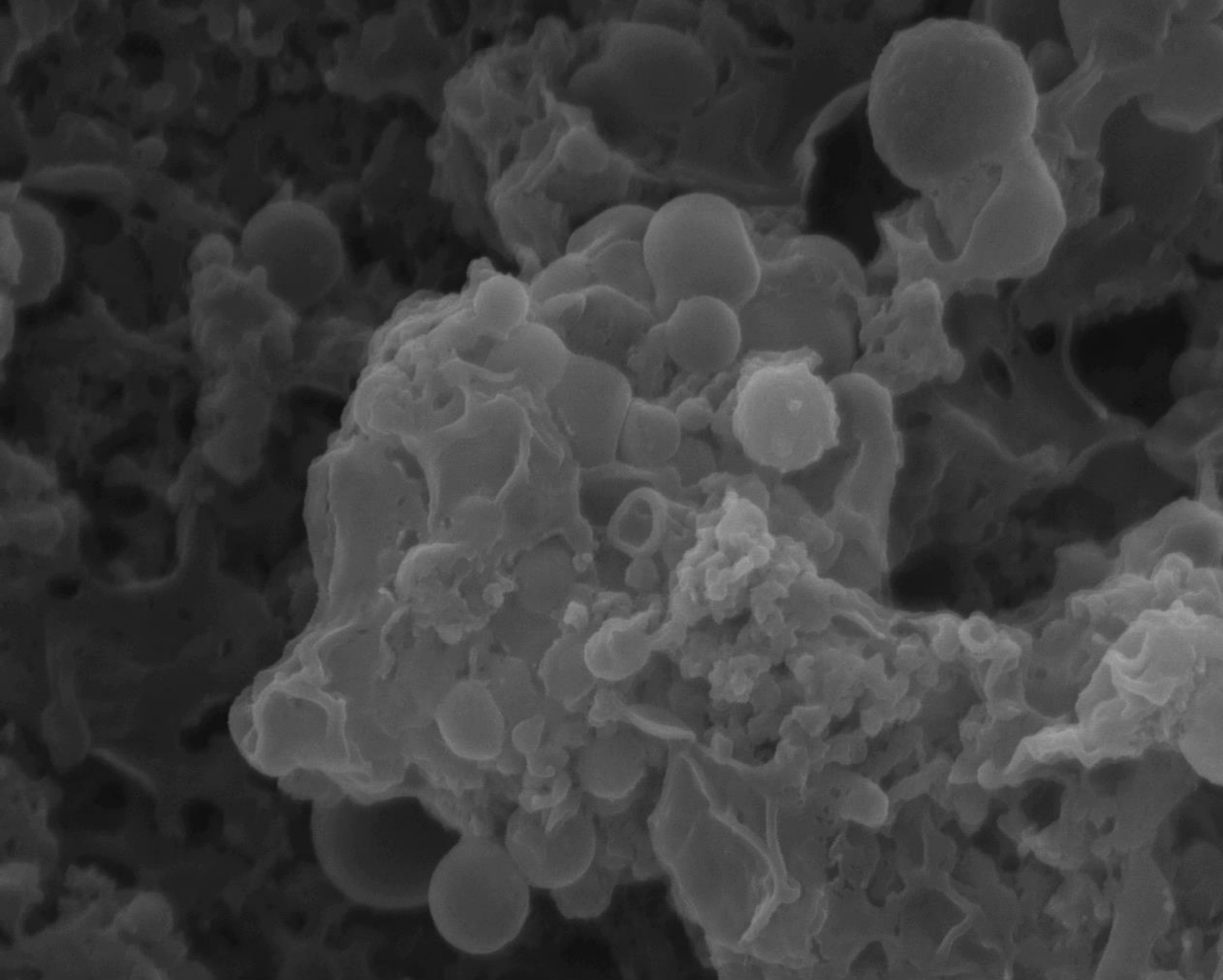
Isolate from spruce needle homogenate 12

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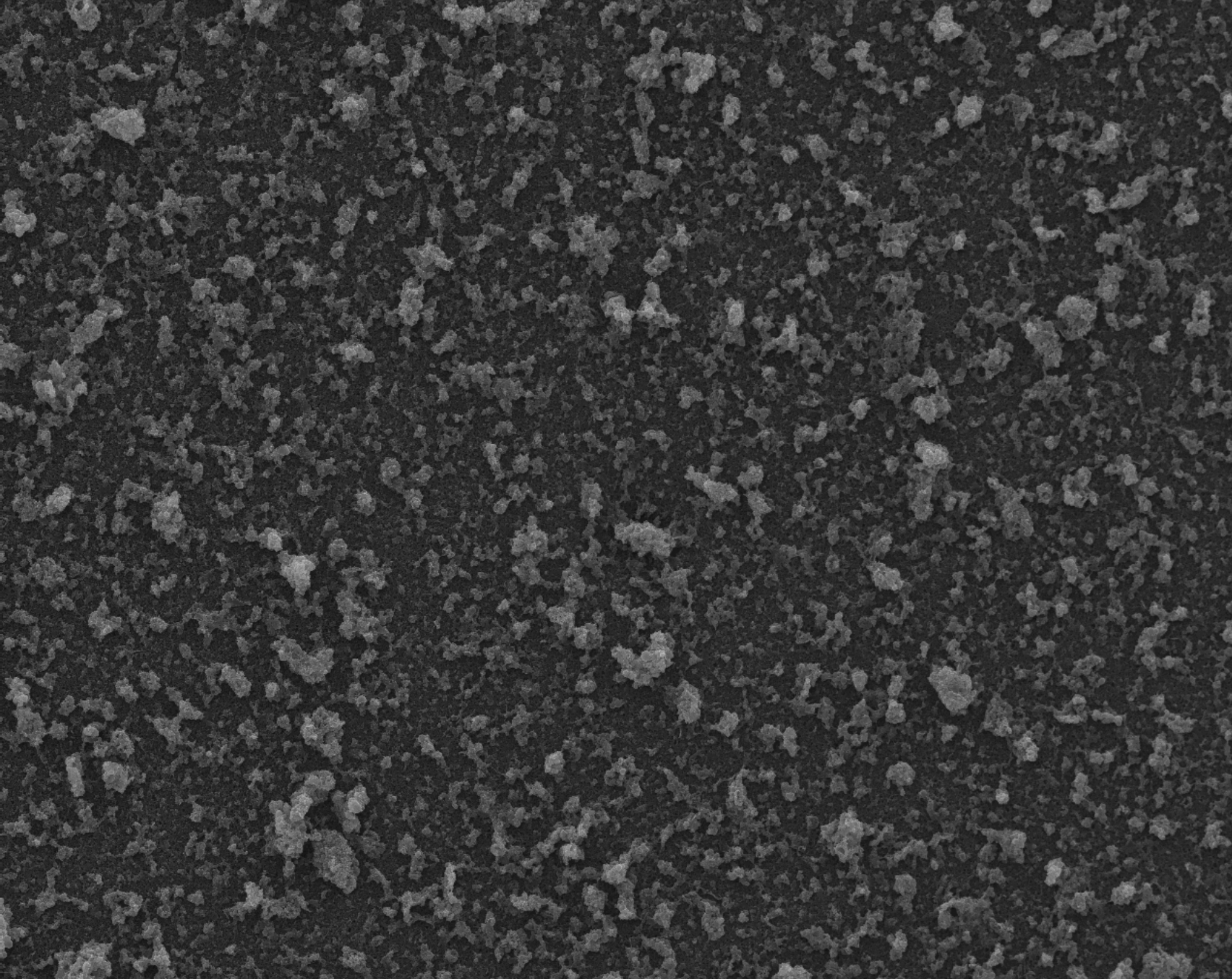
Isolate from spruce needle homogenate 14

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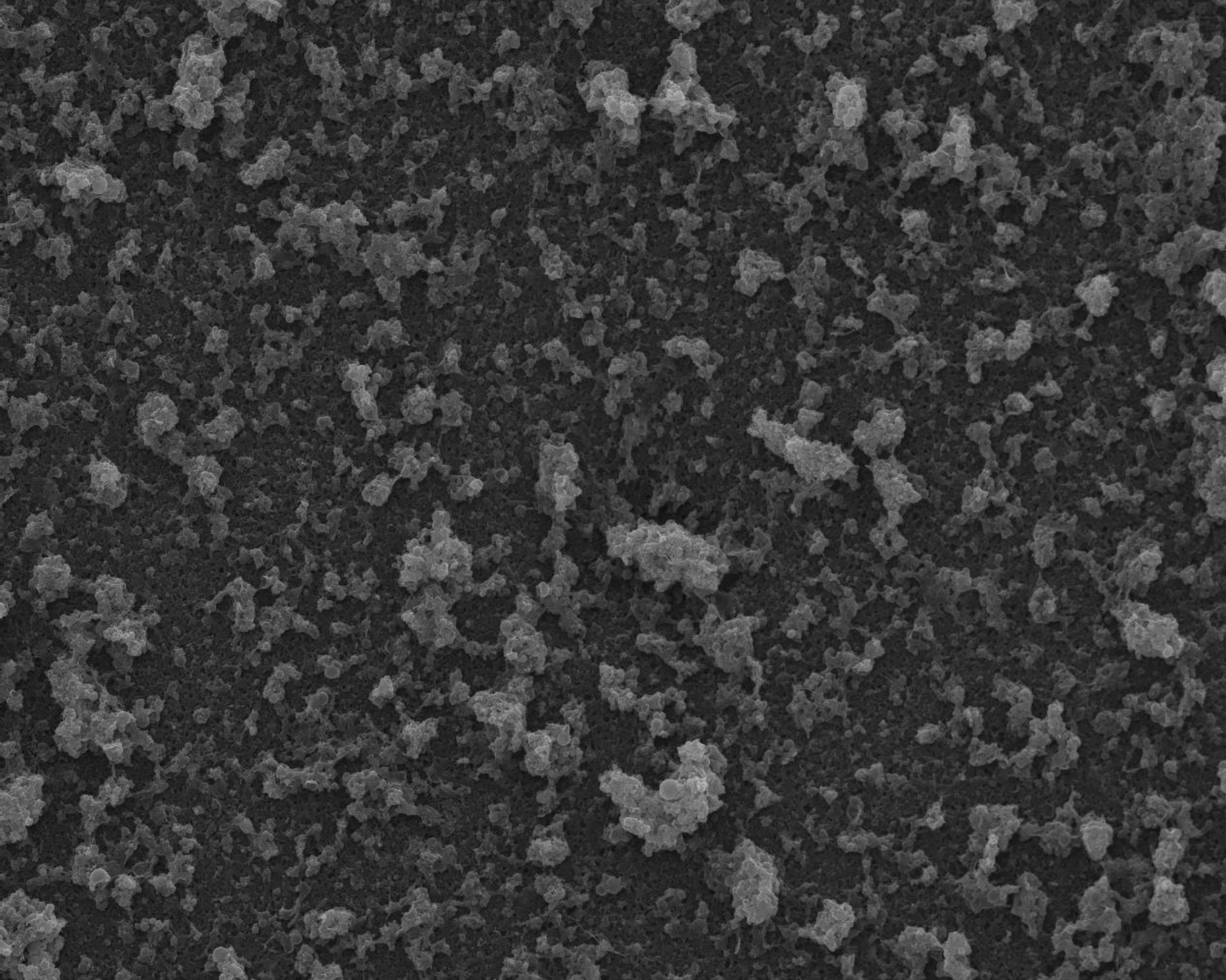
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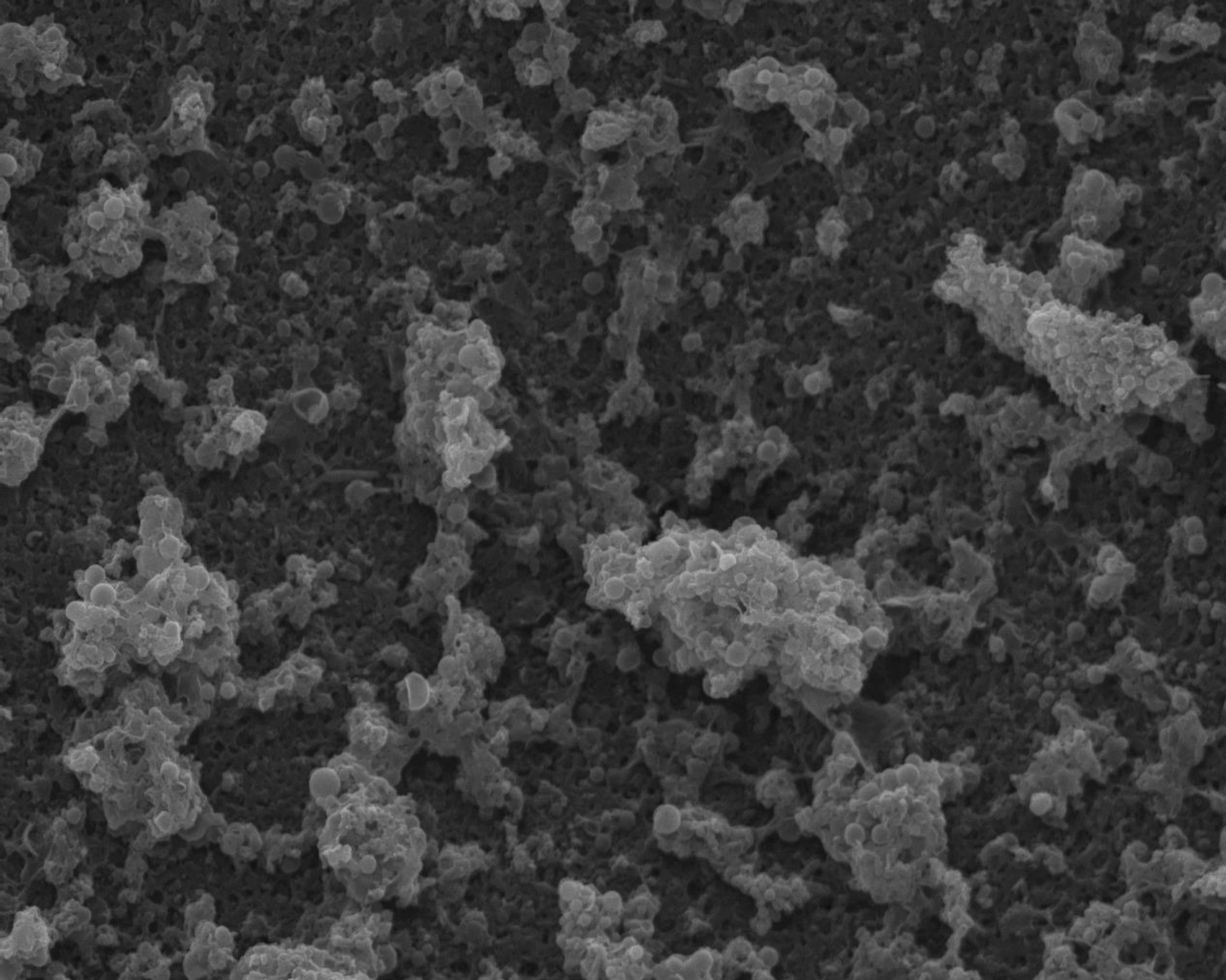
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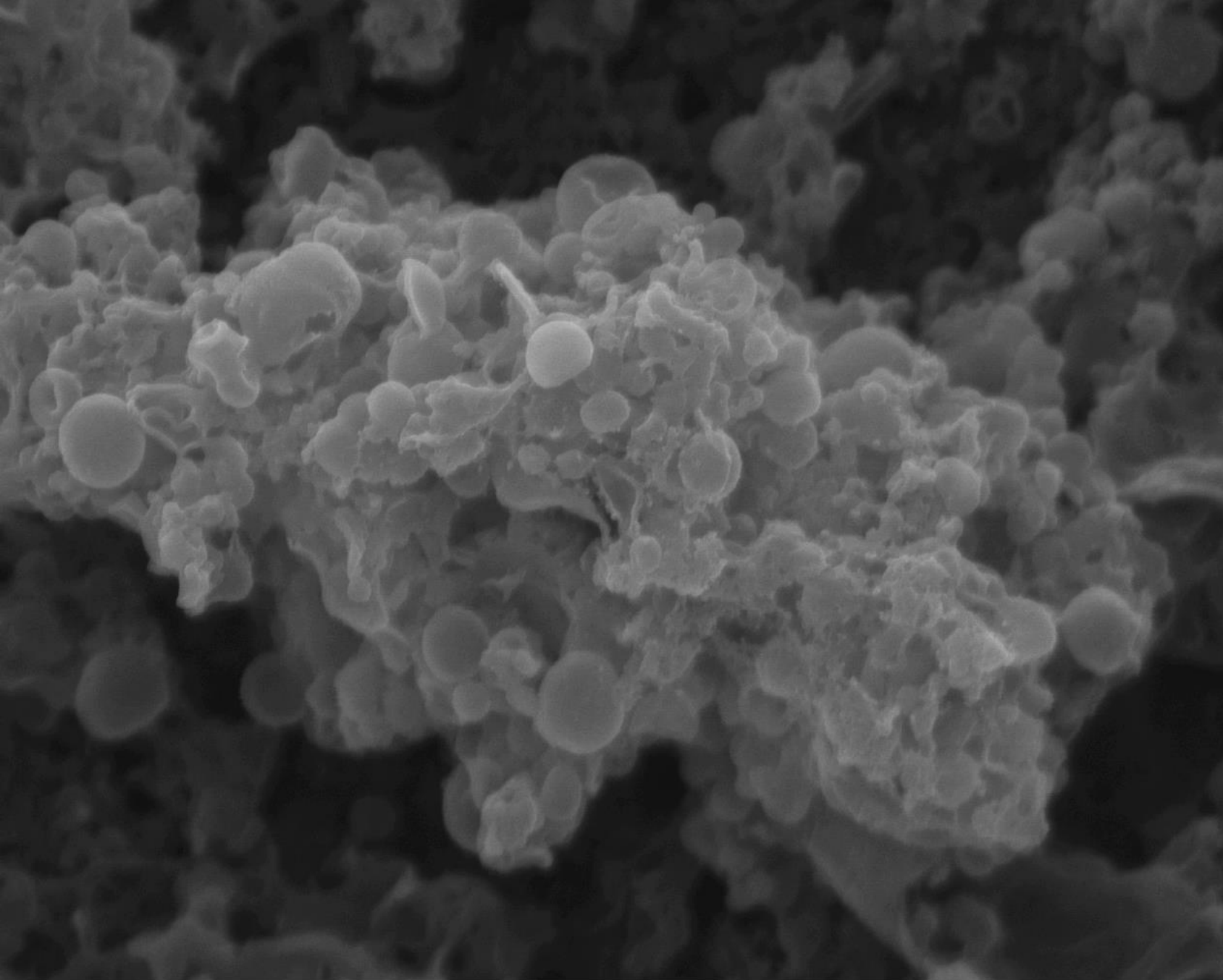
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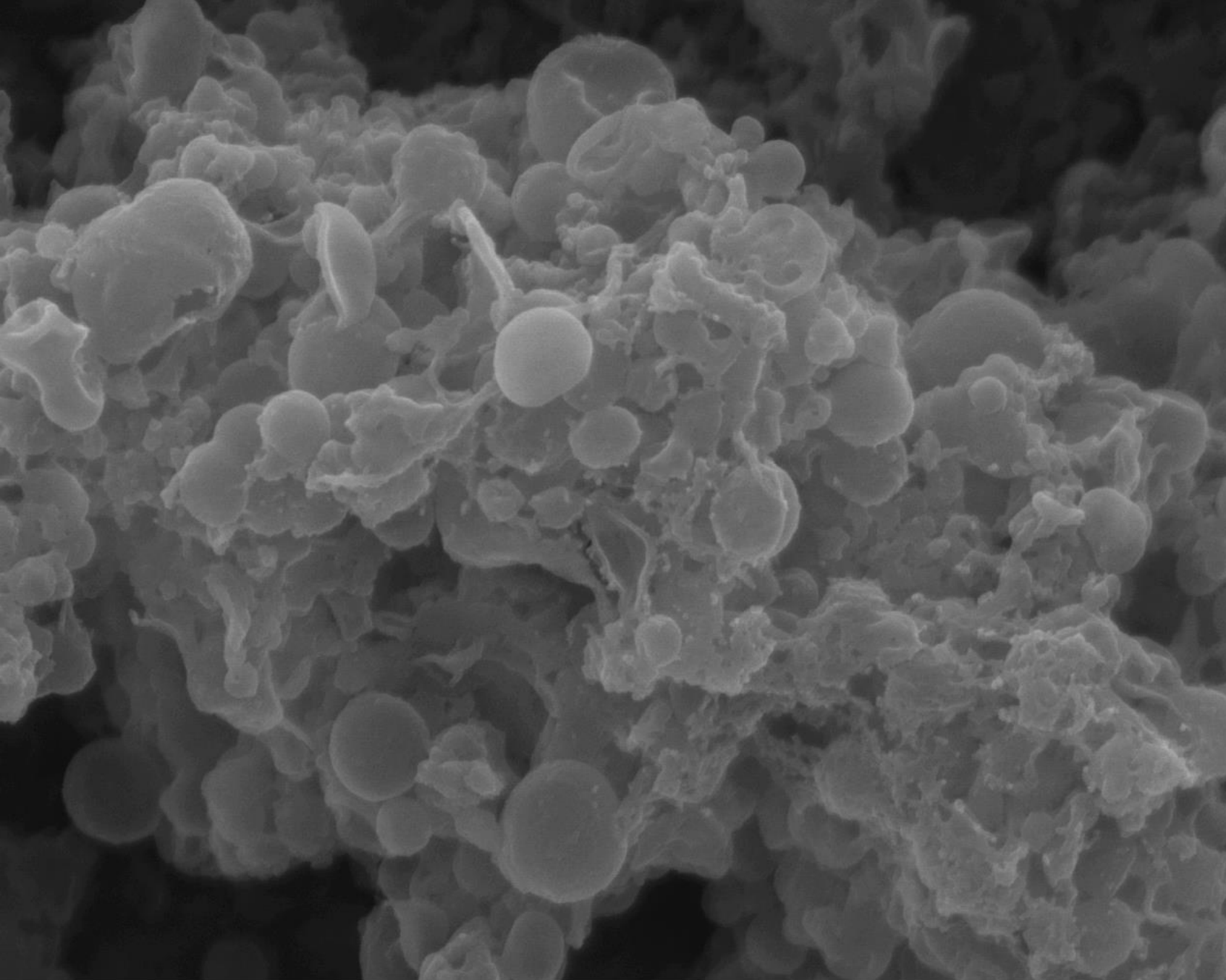
Isolate from spruce needle homogenate 18

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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IMT SEI 15.0kV X65,000 100nm WD 10.0mm

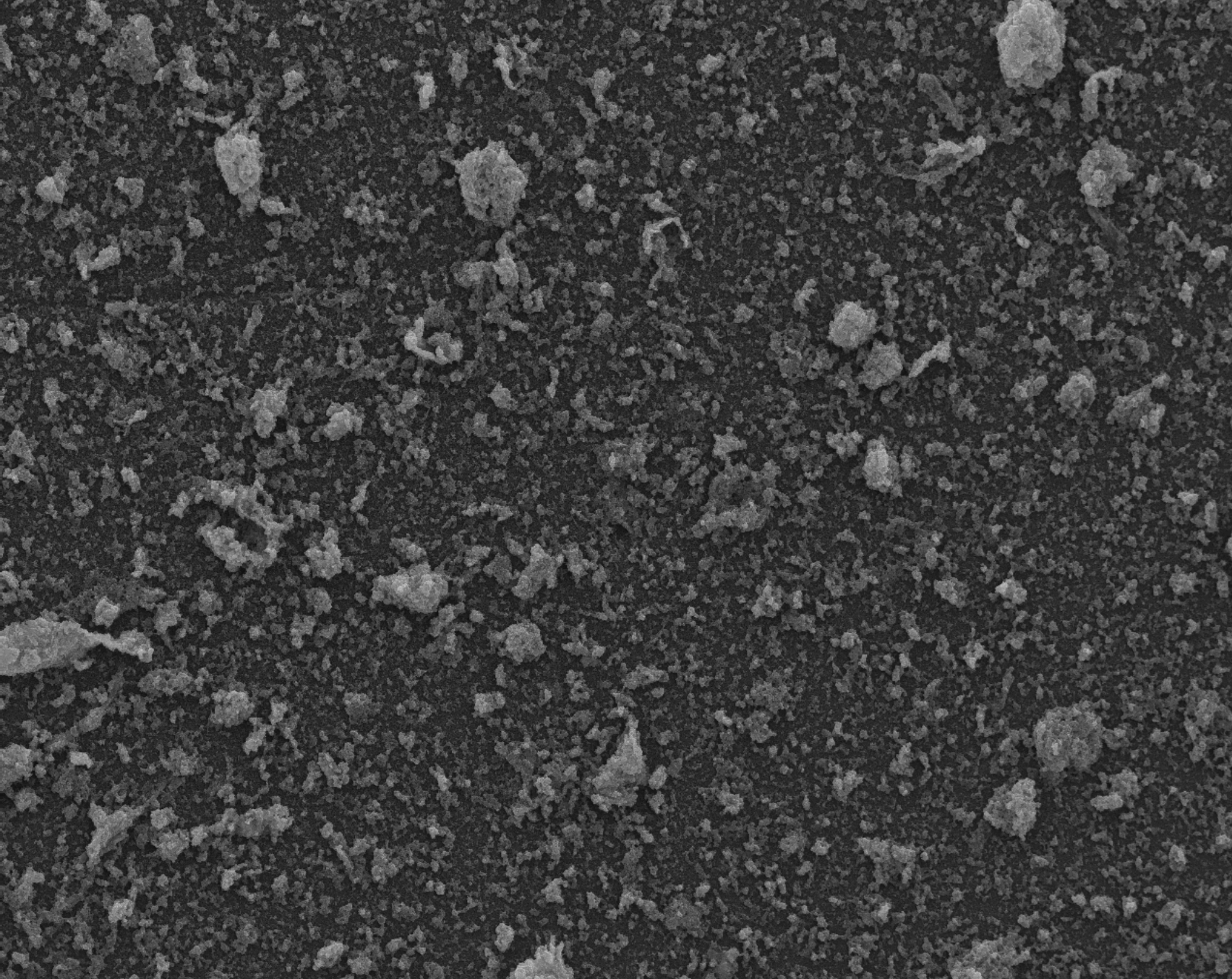
Isolate from spruce needle homogenate 19

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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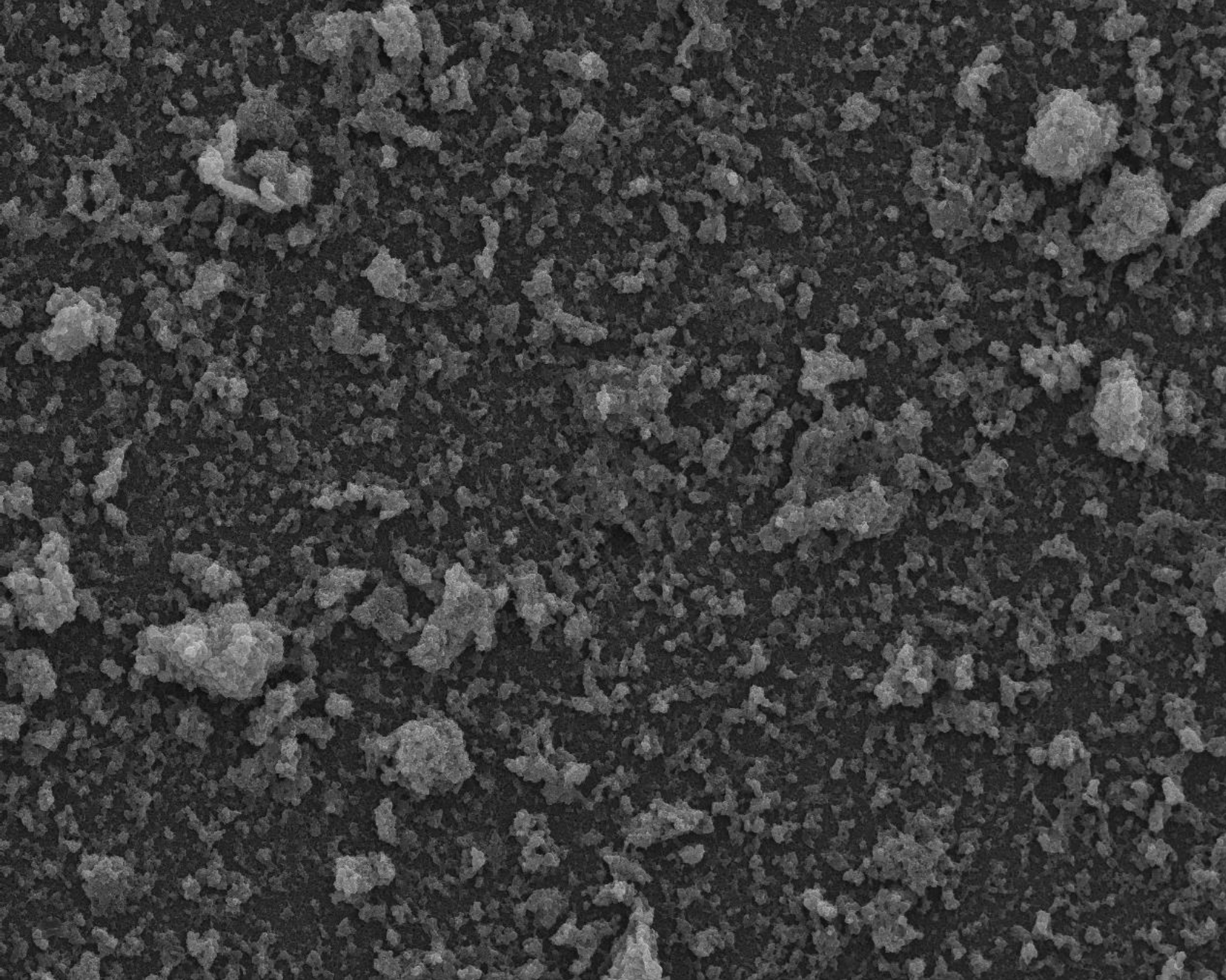


Isolate from spruce needle homogenate 20

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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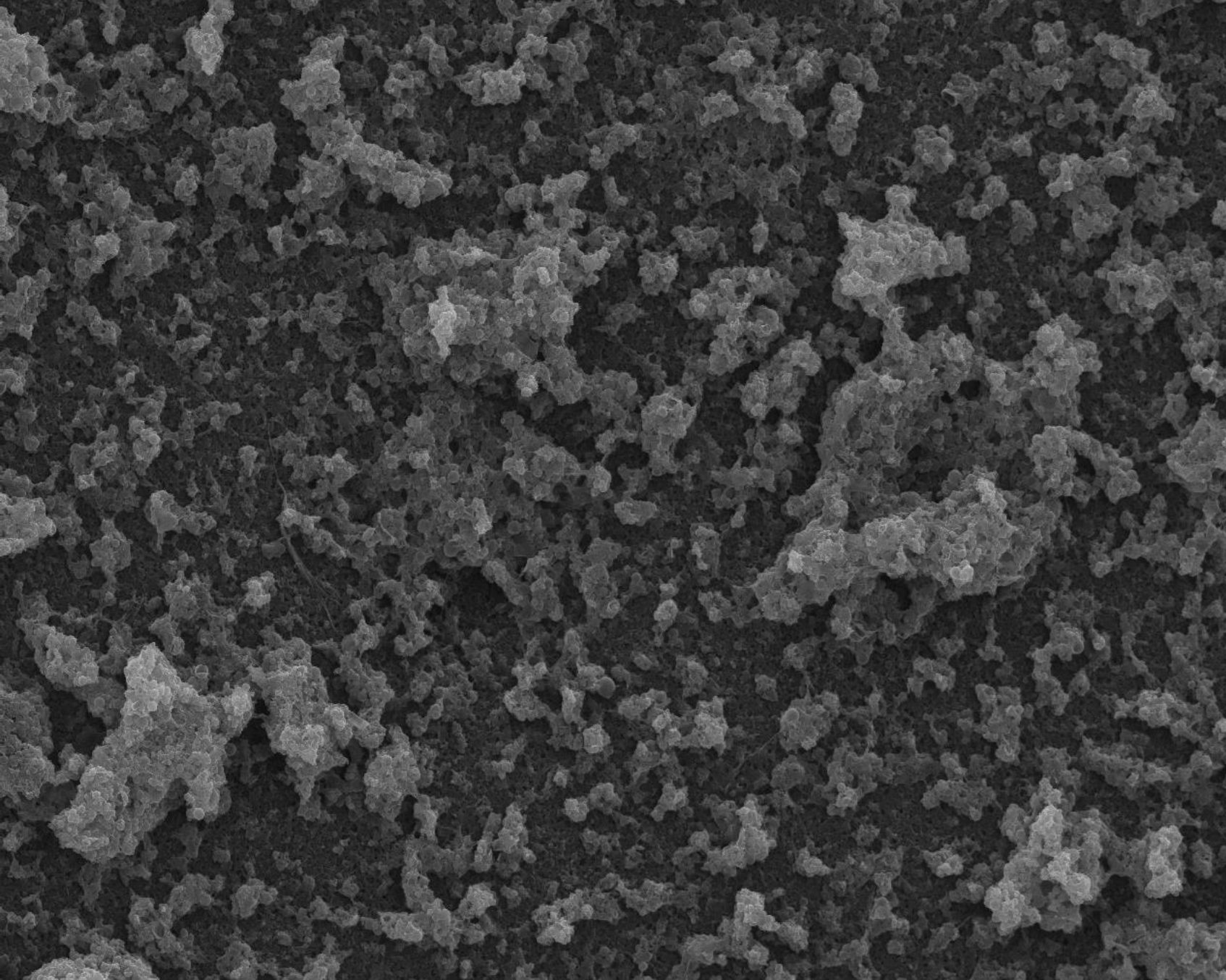
Isolate from spruce needle homogenate 21

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Isolate from spruce needle homogenate 22

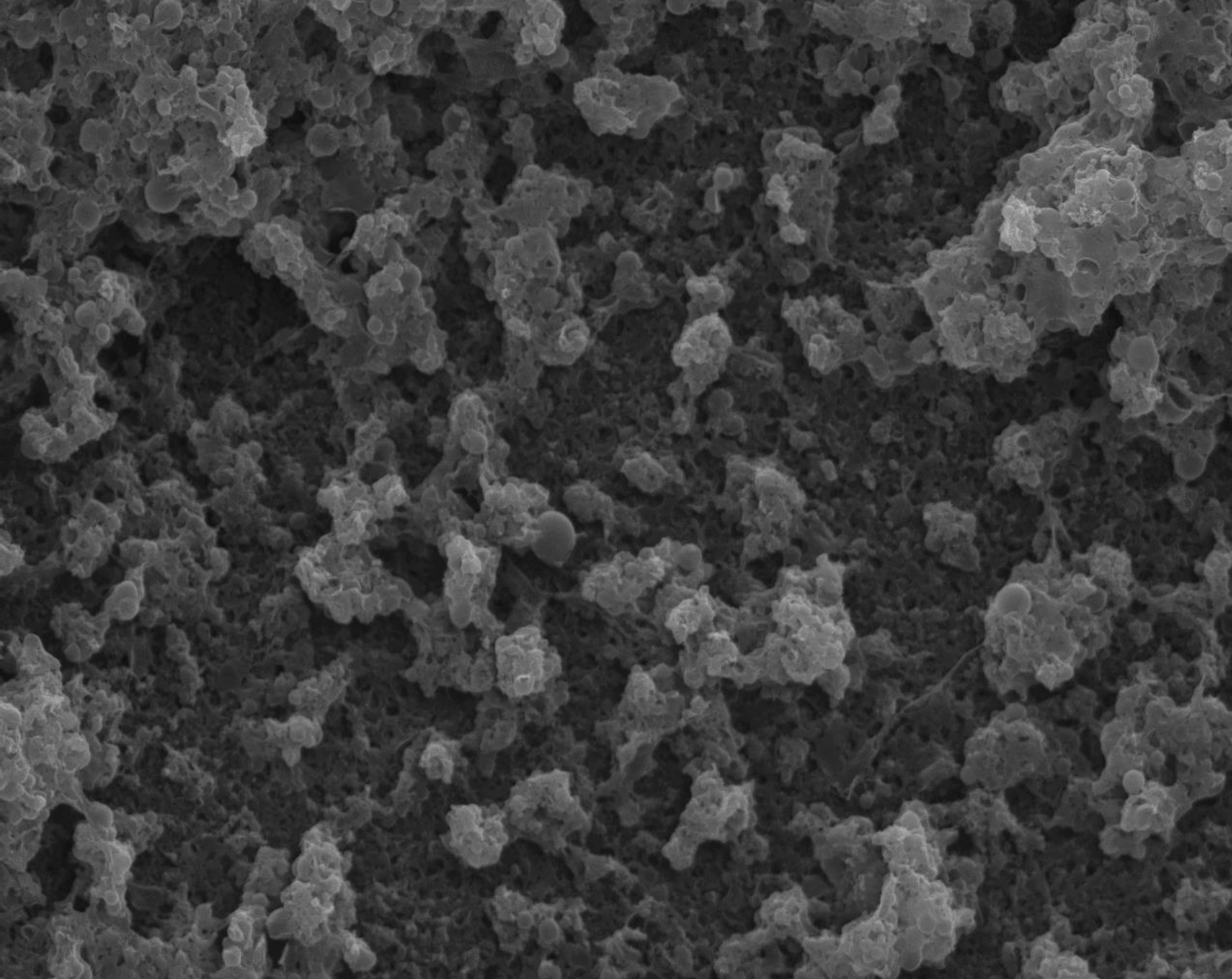
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IMT SEI 15.0kV X2,000 10µm WD 10.0mm



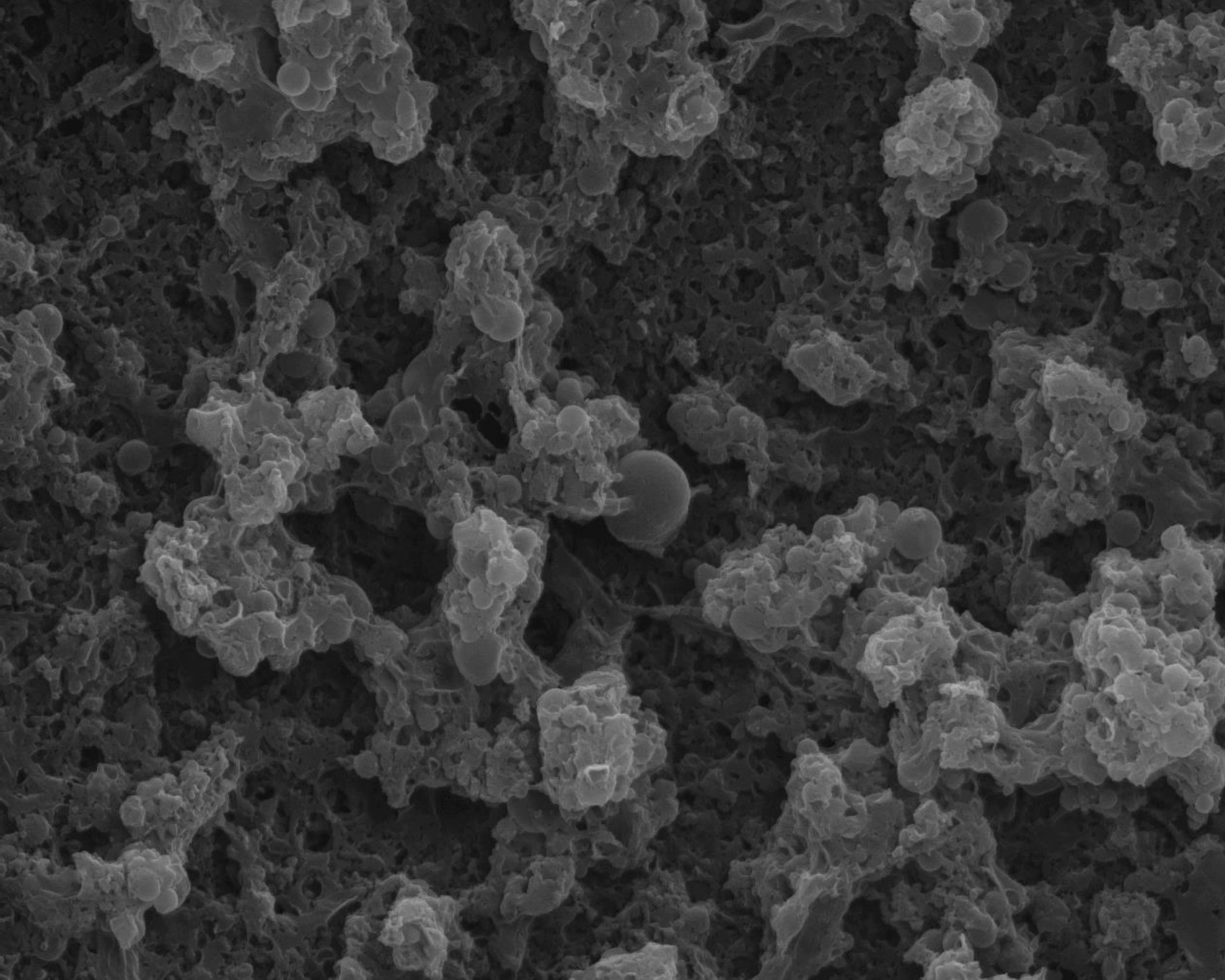
Isolate from spruce needle homogenate 23

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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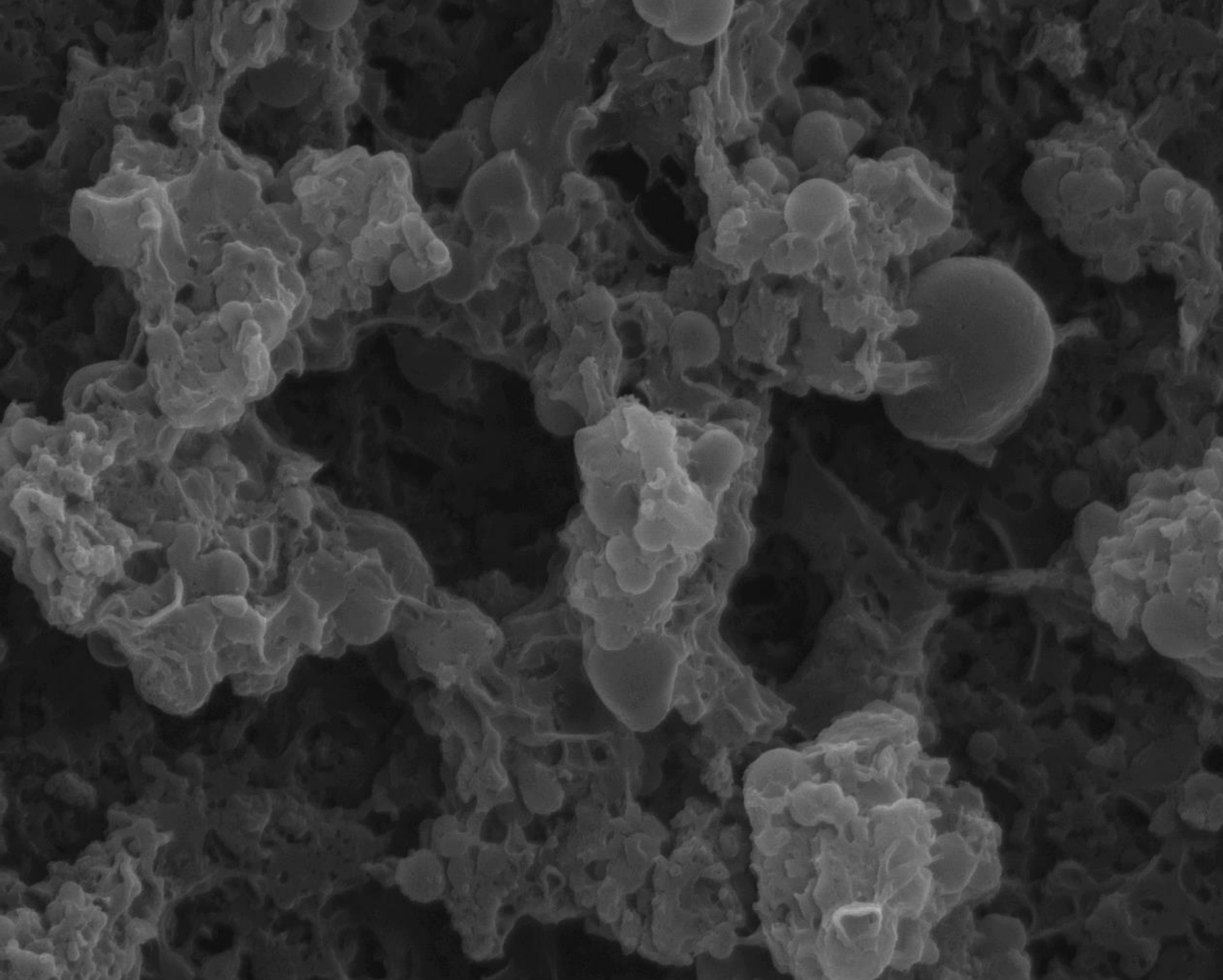
Isolate from spruce needle homogenate 24

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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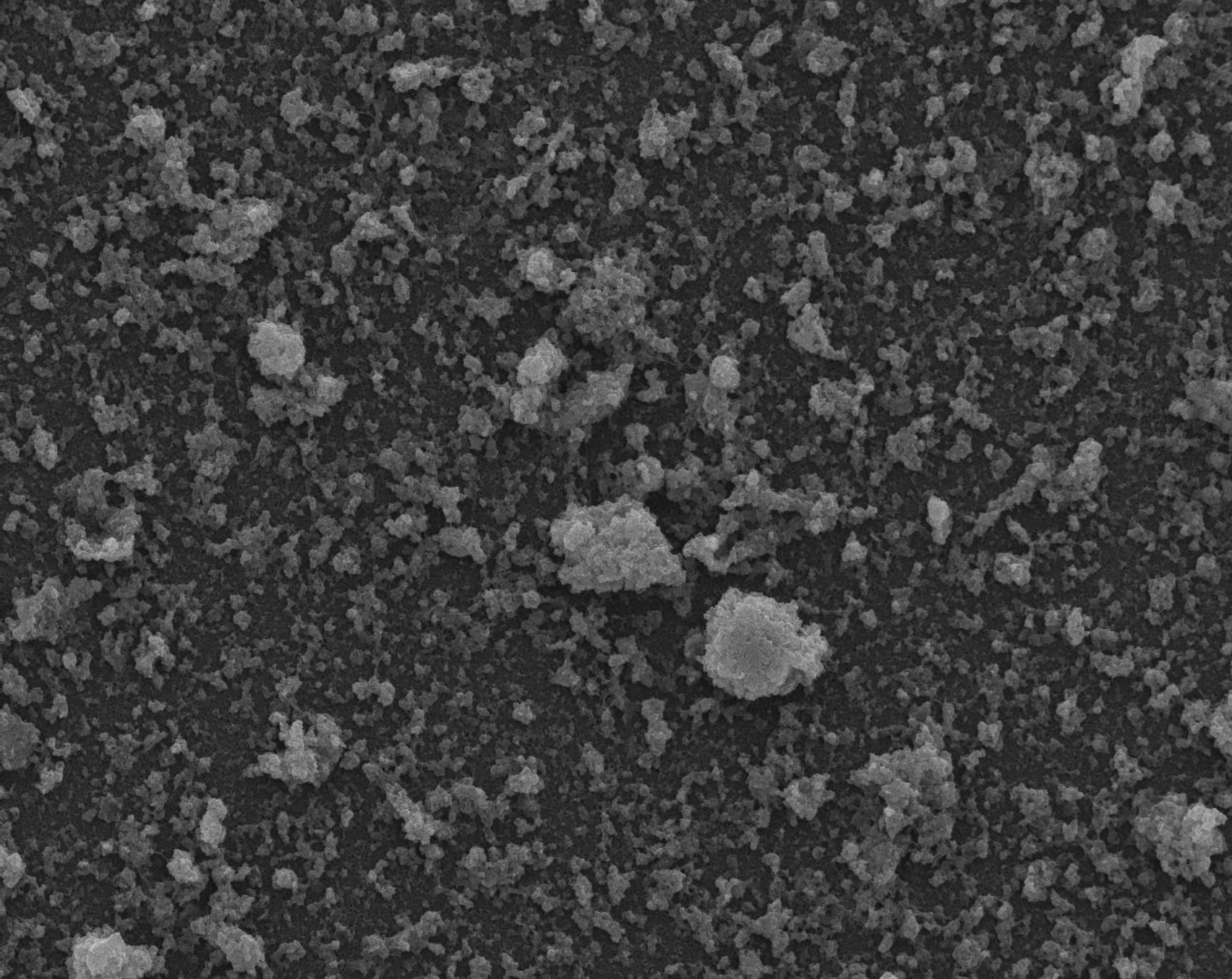
Isolate from spruce needle homogenate 25

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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The samples were fixed with OsO₄ as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. *Int J Mol Sci.* 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO₄ for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).

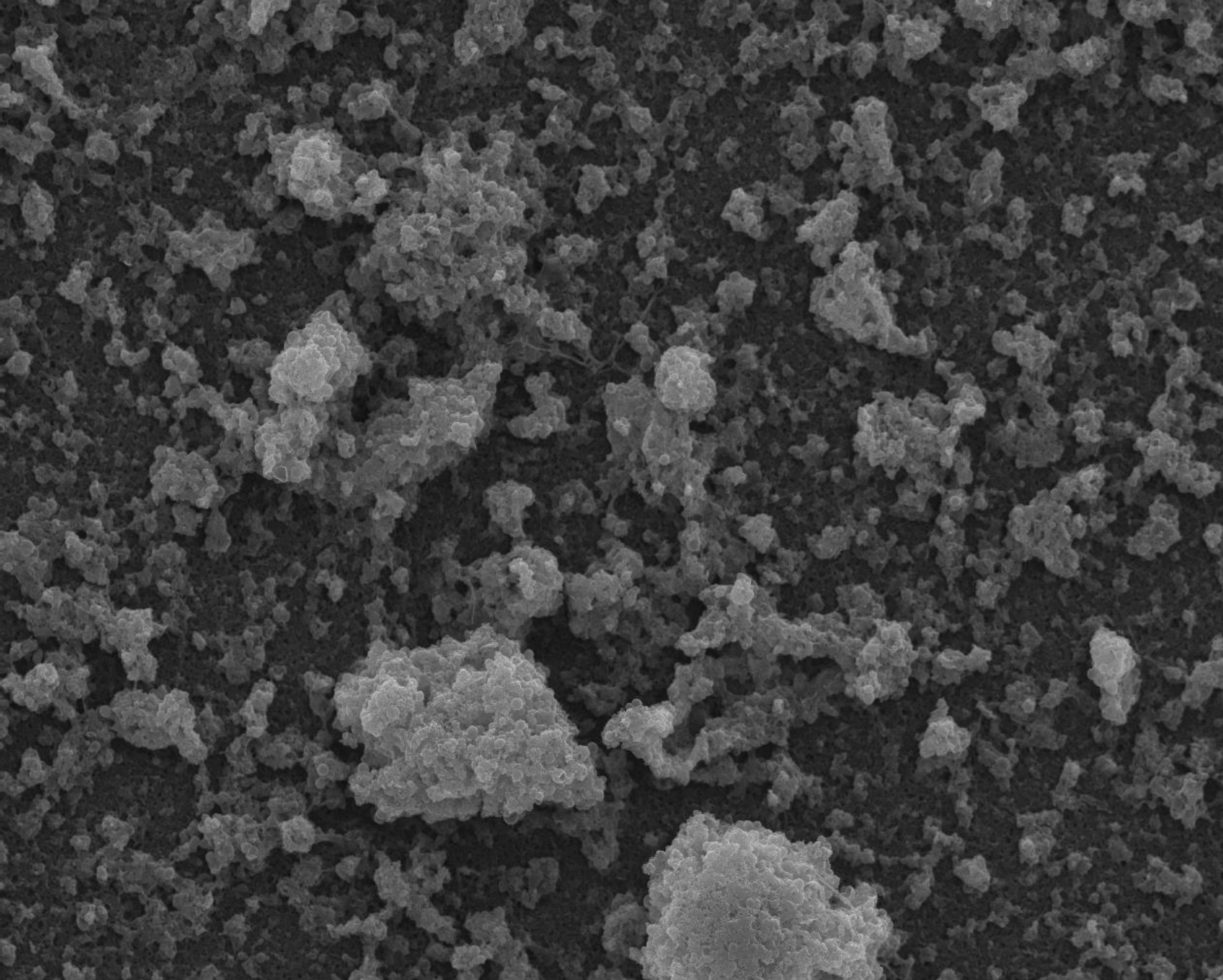


Isolate from spruce needle homogenate 26

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. *Cancers* 2022, 14, 3700. <https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

The samples were fixed with OsO4 as adapted from (Božič, D.; Hočevar, M.; Kisovec, M.; Pajnič, M.; Pađen, L.; Jeran, M.; Zavec Bedina, A.; Podobnik, M.; Kogej, K.; Iglič, A.; Kralj-Iglič, V. Stability of erythrocyte-derived nanovesicles assessed by light scattering and electron microscopy. *Int J Mol Sci.* 2021, 22, 12772. doi:10.3390/ijms222312772). The samples were fixed on 0.05-micron mixed-cellulose-esters' filters (Sterlitech, Auburn, USA). The samples were incubated in 1% OsO4 for 2 h, washed 3-times with distilled water (incubation time 10 min each), then dehydrated in graded series of ethanol (30%, 50%, 70%, 80%, 90% and "absolute", 10 min each; absolute ethanol was replaced 2 times) and hexamethyldisilazane (mixed with absolute ethanol; 30%, 50% and "absolute", 10 min each). Then, the samples were left overnight to dry in air. For examination under JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan), the samples were coated with Au/Pd (PECS Gatan 682).



Isolate from spruce needle homogenate 27

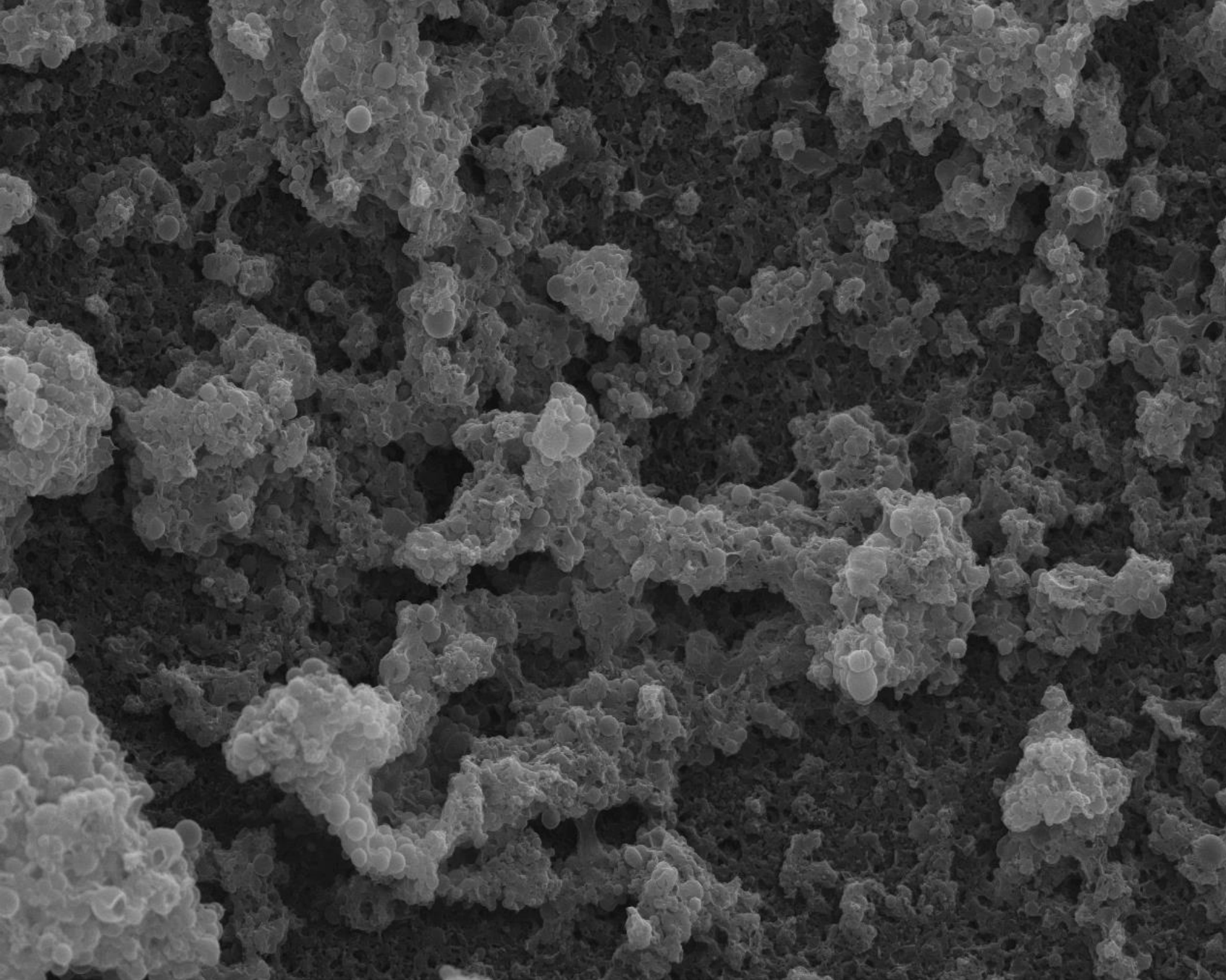
Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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IMT SEI 15.0kV X2,000 10µm WD 10.0mm



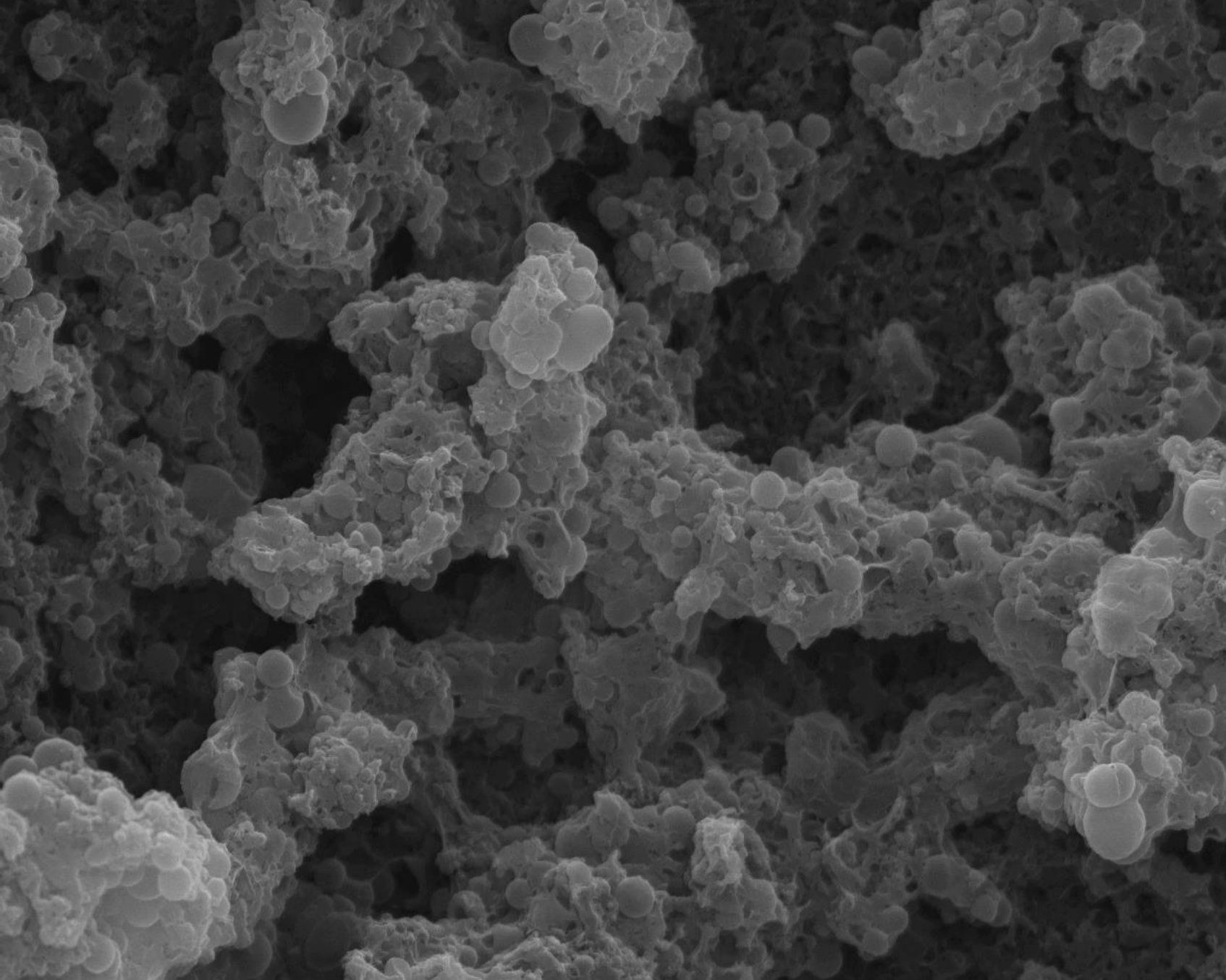
Isolate from spruce needle homogenate 28

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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<https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

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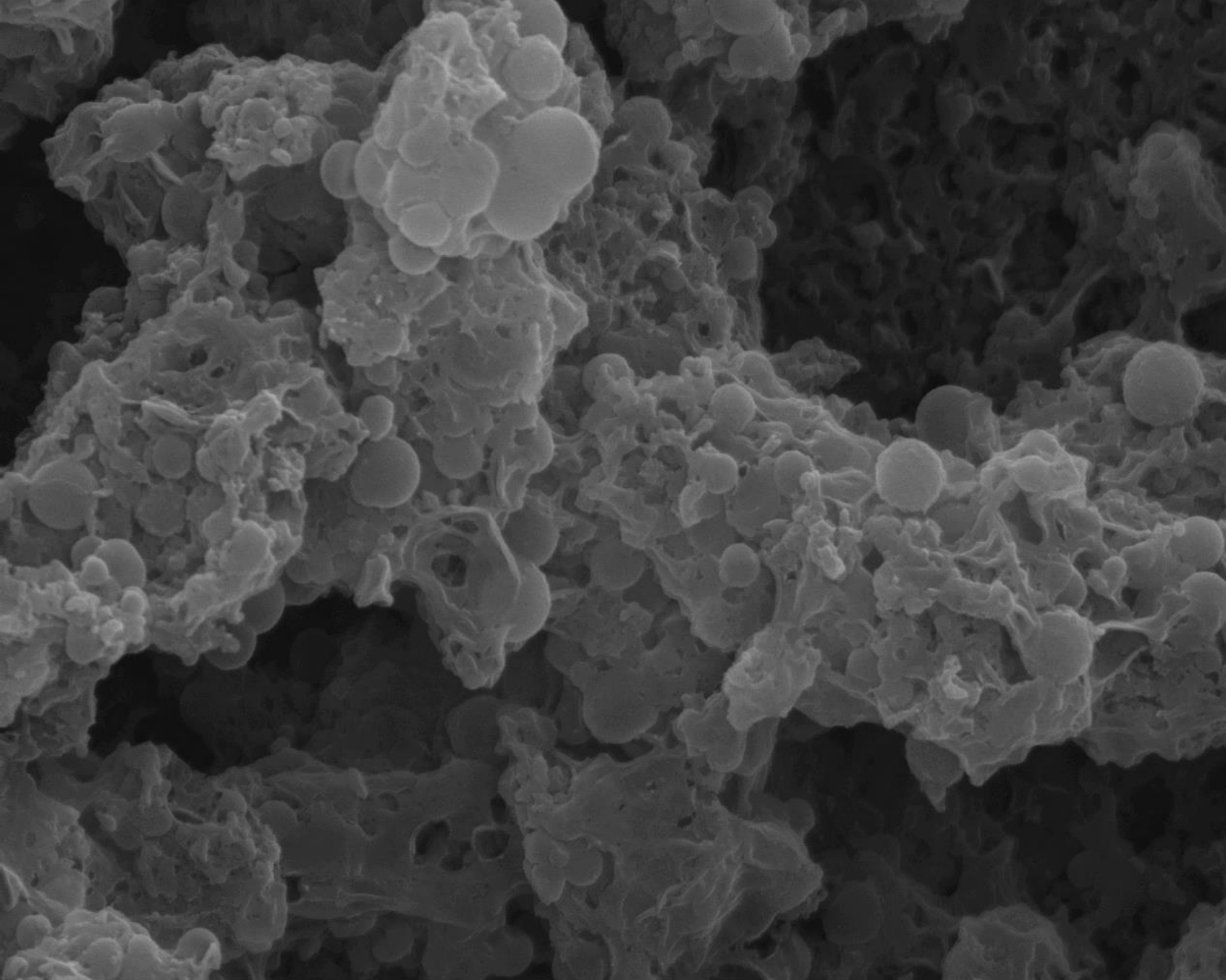
Isolate from spruce needle homogenate 29

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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Isolate from spruce needle homogenate 30

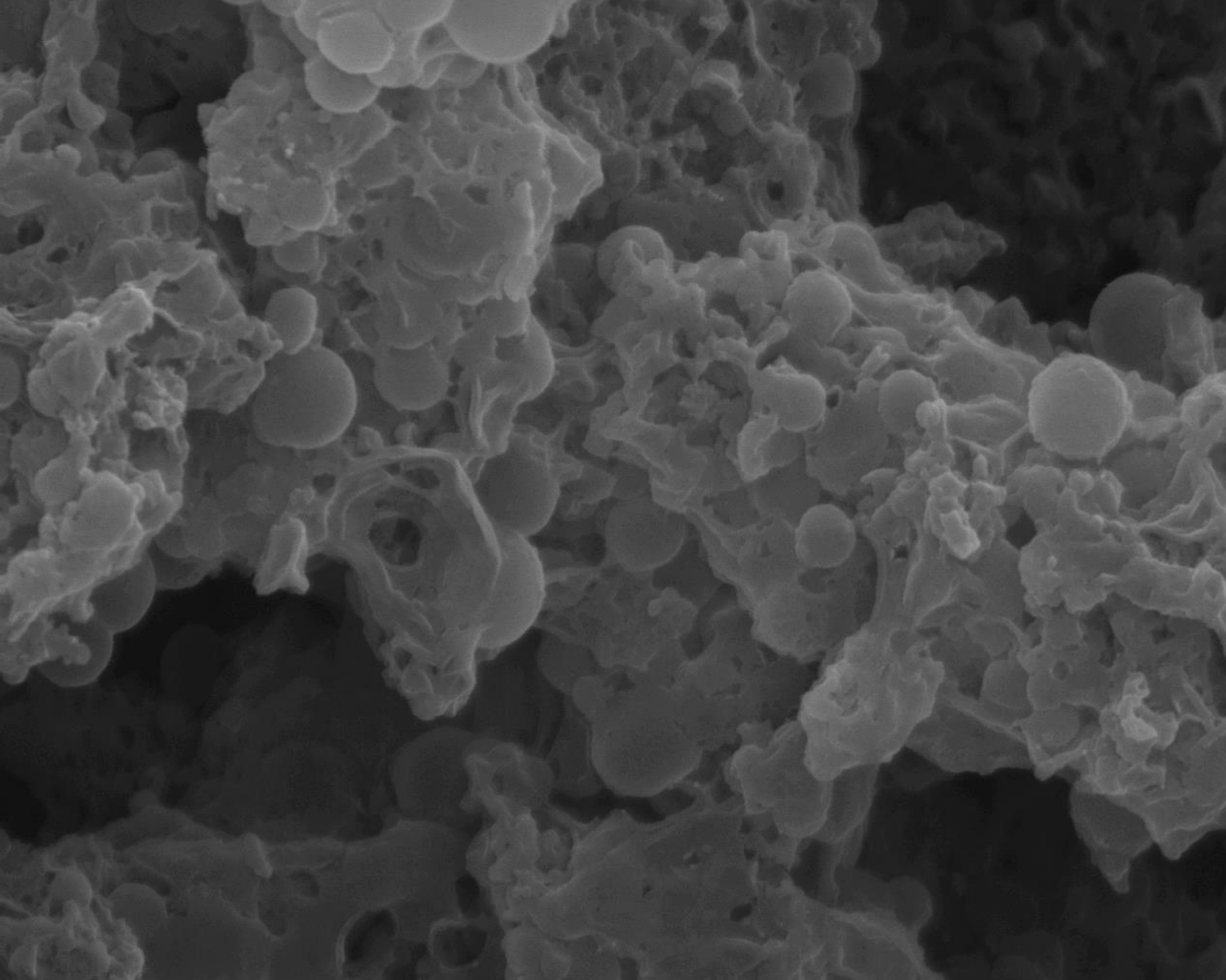
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IMT SEI 15.0kV X20,000 1μm WD 10.1mm



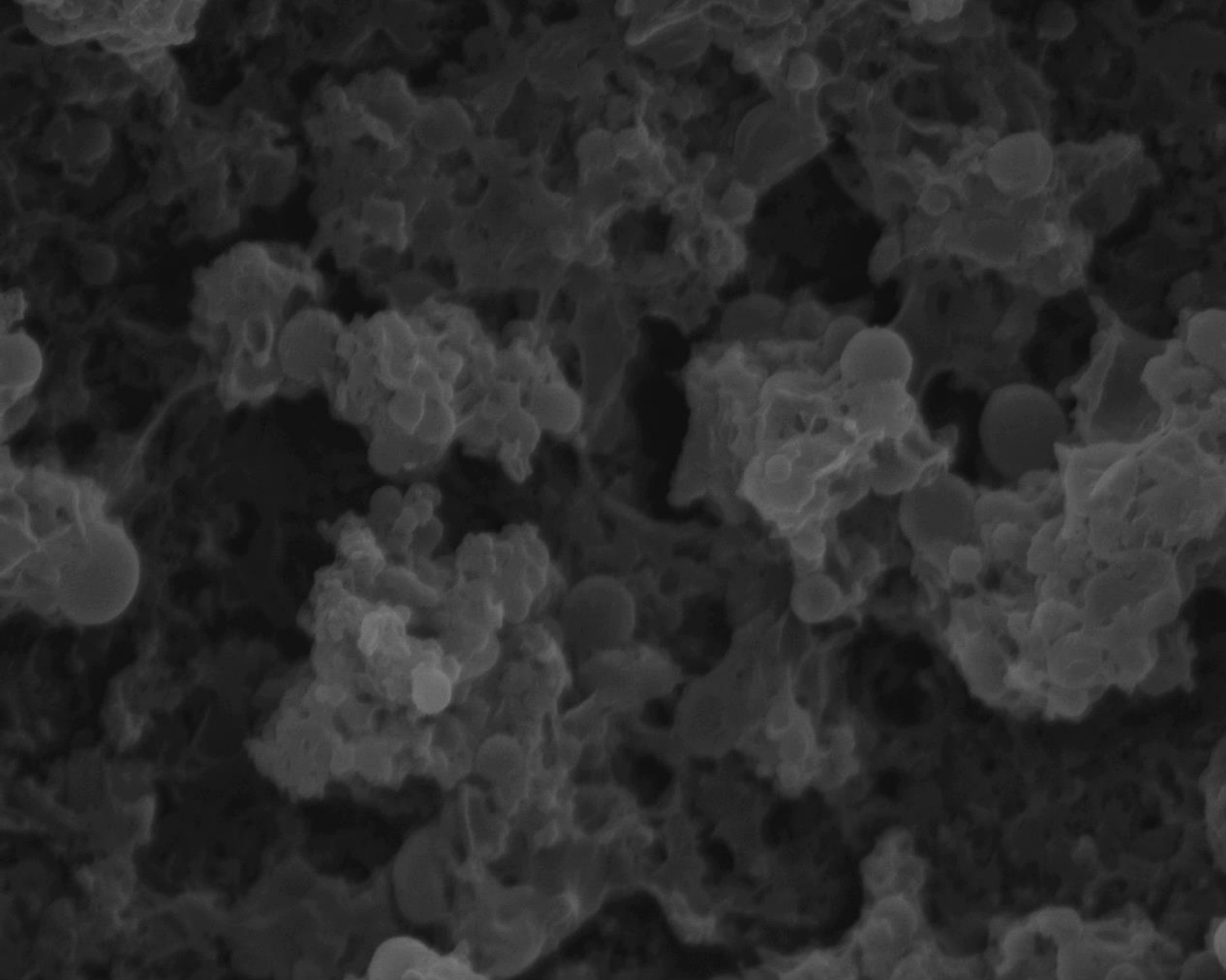
Isolate from spruce needle homogenate 31

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles

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<https://doi.org/10.3390/cancers14153700>). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

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IMT SEI 15.0kV X20,000 1μm WD 10.1mm

Isolate from spruce needle homogenate 32

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