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Datasheet for the decision of 5 May 2026

Case Number: T 0128/25 - 3.3.07

Application Number: 20704206.0

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Title of invention:
PROCEDURE FOR THE PREPARATION OF AN AMNIOTIC MEMBRANE HOMOGENATE BASED ANTIMICROBIAL AGENT

Applicant:
Univerza v Ljubljani

Headword:
PROCEDURE FOR THE PREPARATION OF AN AMNIOTIC MEMBRANE HOMOGENATE BASED ANTIMICROBIAL AGENT/Univerza v Ljubljani

Relevant legal provisions:
EPC Art. 113(1), 111(1), 54, 56
EPC R. 111(2)
RPBA 2020 Art. 13(1), 13(2), 11

Keyword:
Violation of procedure (No)
Admission of a new document (No)
Remittal - (no)
Main request - Novelty of Product-by-Process (No)
Auxiliary requests 1-3 - Inventive step (No)

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Case Number: T 0128/25 - 3.3.07

D E C I S I O N
of Technical Board of Appeal 3.3.07
of 5 May 2026

Appellant: (Applicant)	Univerza v Ljubljani Kongresni trg 12 1000 Ljubljana (SI)
Representative:	Zacco GmbH Bayerstrasse 83 80335 München (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted/electronically transmitted on 11 July 2024 refusing European patent application No. 20704206.0 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman	J. Lécaillon
Members:	D. Boulois
	A. Jimenez

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse European patent application n° 20 704 206.0. The decision was based on 3 sets of claims filed during oral proceedings as main request and auxiliary requests 1-2.

Claims 1, 13 and 14 of the main request read:

"1. A process for the preparation of an antimicrobial agent characterized in that a homogenate of whole human amniotic membrane is used without being further processed by sonication, centrifugation or lyophilisation; the process comprising the steps of:
a) washing a human amniotic membrane in an aqueous solution selected from saline, balanced salt solution and cell culture medium;
b) cutting the human amniotic membrane into pieces and mixing the pieces with an aqueous solution selected from saline, balanced salt solution and cell culture medium to obtain a mixture;
c) homogenizing the mixture obtained in step b) in a homogenizer;
d) filtering the homogenate obtained in step c) through a mesh filter; and
e) formulating the homogenate obtained in step d) as antimicrobial agent or storing the homogenate obtained in step d) under cool conditions before formulating same as antimicrobial agent."

"13. An antimicrobial agent obtainable by the process as defined in any one of claims 1 to 12 for use in the treatment of a bacterial infection."

"14. The antimicrobial agent according to claim 13, for use in the treatment of a bacterial infection in a patient."

In then auxiliary request 1, claims 13 and 14 were deleted and claim 1 had been restricted with regard to point d) as shown in bold: "d) filtering the homogenate obtained in step c) through a mesh filter **wherein the mesh filter has a pore size from 0.5 mm to 1 mm;**".

Claim 1 of then auxiliary request 2 had been further restricted with regard to point c) as shown in bold: "c) homogenizing the mixture obtained in step b) in a homogenizer **wherein the homogenizer is a homogenizer having a motor speed in the range of from 400 to 800W;**".

II. The documents cited during the examination proceedings included the following:

D1: Mukesh K. Yadav *et al.*: "Antimicrobial and Antibiofilm Effects of Human Amniotic/Chorionic Membrane Extract on *Streptococcus pneumoniae*",FRONTIERS IN MICROBIOLOGY, vol. 8, 10 October 2017, pages 1-17,

D2: Qing Guo *et al.*: "A comparison of the effectiveness between amniotic membrane homogenate and transplanted amniotic membrane in healing corneal damage in a rabbit model", ACTA OPHTHALMOLOGICA, vol. 89, no. 4, 2011 pages e315-e319

D3: Yoav P Talmib *et al.*: "Antibacterial Properties of Human Amniotic Membranes", Placenta, vol. 12, 1991, pages 285-288

D4: Majid Zare-Bidaki *et al.*: " Antimicrobial Properties of Amniotic and Chorionic Membranes: A Comparative Study of Two Human Fetal Sacs", J Reprod Infertil, vol. 18, no. 2, 2017, pages 218-224

D5: F.A. Tehrani *et al.*: "The Antibacterial Effect of Low Temperature Stored Amnion on Growth of Escherichia Coli, Staphylococcus Aureus and Pseudomonas Aeruginosa", J BABOL UNIV MED SCI, vol. 20, no. 1, January 2018, pages 13-19

D7: WO2006/094247 A2

D9: CN 107 595 758

D12: "Reusable 0.2/0.45/1/5/10 Micron Sintered Stainless Steel SS 316L Powder Filter Cartridge", pages 1-12, Retrieved from the Internet: URL:https:// www.alibaba.com/product-detail/ Reusable-0-2-0-45-1_62162680981.html

D14: "High Capacity In-Line 0.5 micron Filter", page 1, Retrieved from the Internet: URL:https:// web.archive.org/web/20160118130704/https://www.hplc-asi.com/high-capacity-in-line-0-5-micron filter/

III. According to the decision under appeal, the disclaimer of claim 1 reading "without being further processed by sonication, centrifugation or lyophilisation" was interpreted as in either sonication, or centrifugation, or lyophilisation had to be absent from the process. This meant that three different embodiments were characterised by this disclaimer: one where sonication was absent, a second one where centrifugation was absent and a third one where lyophilisation was absent. D1 had a centrifugation step but no sonication nor

lyophilisation step, meaning it fell within the scope of the first and third embodiment. The language of claim 1 was not considered to be equivalent with the formulation "without being further processed by **any** of the steps of sonication, centrifugation or lyophilisation", which would have excluded D1.

The product claims 13 and 14 of the main request were not novel over D1.

D1 was the closest prior art for the subject-matter of claim 1 of auxiliary request 1. The technical problem over D1 was the provision of a quicker filtration step to obtain an antimicrobial agent. The replacement of the sterile filter in D1 with a well-known mesh filter of 0.5-1 mm pore size was obvious in view of the common general knowledge.

In auxiliary request 2, the additional difference of the homogenization and its related technical effect could be analysed in a partial technical problem independent from the partial technical problem underlying the filtration step, and was the provision of a quicker homogenisation step. This solution was also obvious.

IV. The applicant (appellant) filed an appeal against that decision. With its statement of grounds of appeal, the appellant filed a main request and 3 auxiliary requests. The main request, auxiliary requests 2 and 3 were identical to respectively the main request, auxiliary requests 1 and 2 filed during the examination proceedings.

The new auxiliary request 1 corresponded to the main request with the deletion of the product claims 13 and 14.

V. A communication from the Board, dated 1 December 2025, was sent to the appellant. In it, the Board expressed its preliminary opinion that claims 13 and 14 of the main request lacked novelty over D1 and D2, and that claim 1 of all requests lacked inventive step over D1.

VI. With a letter dated 22 April 2026, the appellant submitted the following evidence:

Annex 2: Additional Experiments comparing hAM homogenates of invention versus those from the prior art

VII. Oral proceedings before the board of appeal took place on 5 May 2026.

VIII. The appellant's arguments can be summarised as follows:

Substantial procedural violation

The reversal of the interpretation of the term "without being further processed by sonication, centrifugation or lyophilisation" in claim 1 at the outset of the oral proceedings before the examining division constituted a substantial procedural violation. It took the applicant by surprise and rendered the preparations for oral proceedings suboptimal.

Admittance of annex 2

These experiments were filed in response to the preliminary opinion of the Board. In view of the thorough experiments, the appellant was unable to conclude these experiments before. There were no opposing party to the proceedings which were negatively affected by the submission.

Main request - Novelty

D1 disclosed a process which comprised a double centrifugation. None of the cited documents disclosed a process for the preparation of an antimicrobial agent characterized in that a homogenate of whole human amniotic membrane was used without being further processed by sonication, centrifugation or lyophilisation, which comprised the steps a) to e). Claim 13, directed to an antimicrobial agent prepared by the process as defined in claim 1, was new over the cited documents for containing bioactive cells and cell fragments that were lost in the processes of the cited documents.

Remittal to first instance

The application should be remitted to the examining division for examination of the compliance with Article 56 EPC.

Auxiliary request 1 - Inventive step

Compared with the process of claim 1, the process of D1 had several differences including freezing in nitrogen, double homogenisation in wet as well as dry conditions, a double centrifugation, a sterile filtration and

finally freezing of the obtained solution. The objective technical problem might be formulated as providing an improved process for preparing an antimicrobial agent from amniotic membrane which is time efficient as well as prevents the loss of active antimicrobial molecules. The skilled person starting from D1 and seeking to solve the objective technical problem might seek to streamline the rather complex process, but would not have eliminated the multiple freeze/thaw cycles, eliminated the double centrifugation as well as the sterile filtration to arrive at a process according to claim 1.

Auxiliary requests 2 and 3 - Inventive step

Claim 1 in both of the auxiliary requests 2 and 3 was inventive at least for the reasons as set forth for auxiliary request 1. Further the limitation to the mesh filter having a pore size of 0.5 to 1 mm distinguished clearly from the sterile filtration of the cited documents, e.g. D1 and D9. This had the technical effect of allowing larger cell fragments, cells and small pieces of tissue to pass the filter such as to minimize loss of bioactive material. Further, the coarser filter size allowed for a quicker process with less clogging and replacement of filters.

Moreover, the skilled person would not start a very intense homogenization to make tiny particles in view of the filtration step.

The additional limitation in auxiliary request 3 of the homogenizer having a low effect motor strengthened even further the retainment of bioactives since it was a much more gentle homogenization. Claim 1 in these two requests was therefore inventive over D9 as well as D1.

IX. Requests

The appellant requested that the decision under appeal be set aside and that the case be remitted to the examining division for further examination on the basis of the main request or of one of the auxiliary requests 1-3 filed with the statement of grounds of appeal.

They also requested that the appeal fee be reimbursed due to a substantial procedural violation and that the experimental data filed with letter dated 22 April 2026 be admitted into the appeal proceedings.

Reasons for the Decision

1. Substantial violation of procedure - Reimbursement of the appeal fees

1.1 The appellant considered that the reversal of the interpretation of claim 1 at the outset of the oral proceedings was in contrast to the interpretation otherwise consistently presented by the examining division during the examination phase, and constituted a substantial violation of procedure.

According to the appellant, the term "without being further processed by sonication, centrifugation and/or lyophilisation" was present in claim 1 of the application as originally filed, and it remained there throughout the examination. The reversal of the interpretation of this term constituted a substantial procedural violation, since the examining division revised their long standing interpretation of the term in claim 1 only at the start of the oral proceedings without any trigger, thereby taking the applicant by

surprise and rendering the preparations for oral proceedings suboptimal for targeting the examining division's preliminary opinion, which was based entirely on **a different rejection** and **a different document.**

1.2 The Board does not share the appellant's view.

1.2.1 It is true that the examining division reverted its opinion during oral proceedings with regard to the interpretation of the disclaimer of claim 1. The Board recalls however that provisional opinions are not binding and that a party should be prepared to this situation. **The reversal of an opinion during oral proceedings cannot indeed constitute a surprise, let alone a procedural violation.**

1.2.2 In the present case, it is also evident that all relevant documents and all arguments regarding novelty and in particular inventive step were on file and presented in the preliminary opinions and communication of the examining division.

Novelty of claim 1 over *inter alia* the documents D1 and D2 was assessed in the preliminary opinions dated 14 February 2024 and 27 May 2024, and even if the preliminary opinion of the examining division did not discuss the novelty of the product claims 13 and 14, a discussion on this point was to be expected during the oral proceedings.

The preliminary opinion of the examining division with regard to inventive step over D9 was negative in both communications dated 14 February 2024 and 27 May 2024, with regard to the process claim 1 or the products claims 13 and 14. Even if the examining division chose

finally D1 as closest prior art instead of D9 during the oral proceedings, the arguments regarding inventive step in the preliminary opinions were the same as those in the decision, and D1 was already cited to show the obviousness of the claimed subject-matter. The communications also pointed out under point 6.3 that neither the process of claim 1 nor the antimicrobial agent for use in the treatment of bacterial infection was inventive over *inter alia* D1 as such, and, therefore, the choice of D1 as closest prior art could not constitute a surprise.

1.2.3 Finally, it appears clear that the appellant was given an opportunity to respond to the argument and objections of the examining division during the oral proceedings, since the appellant filed a new main request, as well as two new auxiliary requests (see the minutes of the oral proceedings).

1.2.4 It follows that the decision under appeal is based on known documents and grounds on which the applicant had an opportunity to comment, according to the requirements of Article 113(1) EPC and Rule 111(2) EPC. The Board thus cannot see any procedural violation, let alone a substantial one, which could justify reimbursement of the appeal fee.

At last, one of the conditions for reimbursement of the appeal fee is that the appeal is allowable, at least in part (Rule 103(1)(a) EPC). This condition is neither fulfilled in light of the conclusions reached by the Board with regard to novelty and inventive step of all requests on file; the Board notes furthermore that it arrives to the same conclusions than the examining division with regard to novelty and inventive step, even with a different interpretation of the disclaimer

in claim 1, showing that said interpretation has no incidence on the final result.

2. Admittance of Annex 2

2.1 This document has been filed by the appellant with its letter of 22 April 2026. It discloses the results of additional experiments conducted by the appellant upon receipt of the communication of the Board of Appeal on 1 December 2025 giving its preliminary opinion.

According to the appellant, these data directly address the novelty assessment of claims 13 and 14 of the main request, and the inventive step assessment of claim 1 in the main request.

2.2 Annex 2 provides a comparison between three preparations of human amniotic membrane (hAM), namely between:
(i) a crude hAM homogenate prepared through the process of the invention,
(ii) a sterile filtered hAM homogenate, wherein the same homogenate was prepared after additional sterile filtration through a 0.22 µm filter (as in D1), and
(iii) the soluble hAM extract obtained from the homogenate after centrifugation.

Annex 2 constitutes a clear amendment to the appellant's case and Articles 13(1) and 13(2) RPBA apply.

According to Article 13(2) RPBA 2020, any amendment to a party's case after notification of a summons to oral proceedings shall, in principle not be taken into account, unless there are exceptional circumstances, which have been justified with cogent reasons by the

party concerned. Such exceptional circumstances might for instance reside in objections formulated for the first time in the provisional opinion of the Board.

In the present case, the Board mentioned in its preliminary opinion in the context of the assessment of novelty and inventive step that the product claims 13 and 14 were not novel, and that the process claim 1 of all requests was not inventive. Hence, the Board cannot seen any new point raised in this context, since the same conclusions were also reached by the examining division in its decision with essentially the same arguments.

Consequently, the points raised by the Board in its communication were already discussed during the examination proceedings and present in the decision of the examining division, and there are therefore no exceptional circumstances justifying the filing of new documents at such late stage of the appeal proceedings. For this reason alone, Annex 2 is not admitted into the appeal proceedings.

Moreover, in its letter of 22 April 2026, the appellant merely stated that "the data directly address the novelty assessment of claims 13 and 14 of the main request and the inventive step assessment of claim 1 in the main request" and that “the data shed light on contested technical effects”, without providing any explanation as to the relevance of the experimental data for the assessment of novelty and inventive step. Instead, the appellant left it entirely to the Board to identify the alleged effect demonstrated by the data and to determine how, if at all, that effect might impact the inventive step assessment set out in the Board's preliminary opinion. Such a submission does not

constitute the justification required under Article 13(1) RPBA for the admission of an amendment filed after the statement of grounds of appeal.

Moreover, the Board considers that the experiments of Annex 2 are indeed questionable with regard to their relevance for the assessment of novelty and inventive step and open new points of discussions, therefore adding also complexity to the case. For instance, the experiments do not provide a valid comparison with the process disclosed in D1, since it uses a centrifugation step at 1000 × g for 10 min at 24°C, which does not correspond to the centrifugation parameter disclosed in D1, namely twice at 12000 at 4°C. Annex 2 is also silent with regard to the homogenization speed and conditions used in its process according to the invention and which appears essential in the assessment of novelty and inventive step; the document indeed merely generally states that "the tissue was then homogenized for 3–4 min using the RH16 blender" without any further specification . This document does neither respond to the essential points mentioned by the examining division in its decision or by the Board in its preliminary opinion, namely the absence of characterizing features in the claims of the homogenization and/or filtration steps; in this context, it does anyway not appear possible to extrapolate the experimental results of Annex 2 to the claimed process steps.

Consequently, the Annex 2 is not admitted into the appeal proceedings (Articles 13(1),(2) RPBA).

3. Main request - Novelty of claims 13 and 14

3.1 Claim 13 has been drafted under the form of a product-by-process claim, hence a product obtainable by the process of any of claims 1-12, without any additional product features, for use in the treatment of a bacterial infection.

The claimed antimicrobial agent is in particular characterized by the "product-by-process features" of claim 1, comprising the essential following preparation steps:
a) **washing** a human amniotic membrane in an aqueous solution selected from saline, balanced salt solution and cell culture medium;
b) **cutting** the human amniotic membrane into pieces and **mixing** the pieces with an aqueous solution selected from saline, balanced salt solution and cell culture medium to obtain a mixture;
c) **homogenizing** the mixture obtained in step b) in a homogenizer;
d) **filtering** the homogenate obtained in step c) through a mesh filter; and
e) **formulating** the homogenate obtained in step d) as antimicrobial agent or **storing** the homogenate obtained in step d) under cool conditions before formulating same as antimicrobial agent.

Accordingly, in order to establish novelty, it has to be verified that the process features of the "product by-process" claim are such that the resulting product is influenced by them in a way that it can be distinguished from the products of the prior art produced by a different process. In other words, the process features can only contribute to the novelty of the claimed agent insofar as they give rise to a

distinct and identifiable characteristic of the product. In such a case, where a process feature is the only feature allegedly conferring novelty to a product, the burden of proof for showing the fact that the process feature results in a distinct and identifiable characteristic of the product is on the patent applicant.

3.2 Claim 1 comprises furthermore a disclaimer reading "without being further processed by sonication, centrifugation, or lyophilisation", which interpretation was debated during the examination proceedings .

This disclaimer has been interpreted by the examining division as in **either sonication, or centrifugation, or lyophilisation** has to be absent from the process, which means that three different embodiments are characterised by this disclaimer: one where sonication is absent, a second one where centrifugation is absent and a third one where lyophilisation is absent. According to the examining division, the language of claim 1 was not considered to be equivalent with the formulation "without being further processed by **any** of the steps of sonication, centrifugation or lyophilisation".

The appellant considers that claim 1 clearly excluded the presence of all three process steps. There is therefore no further process by any of sonication, centrifugation or lyophilisation.

The Board agrees with the appellant and considers that the disclaimer of claim 1 **excludes explicitly and clearly any of sonication, centrifugation or lyophilisation**. Accordingly, the product of claims 13

and 14 has been obtained via a process not involving any of sonication, centrifugation or lyophilization.

3.3 The examining division came to the conclusion that claims 13 and 14 were not novel over D1.

3.3.1 D1 relates to the antimicrobial and antibiofilm effects on Streptococcus pneumoniae of a human amniotic/ chorionic membrane extract.

The antimicrobial membrane extract of D1 was prepared from human amniotic membranes through the following steps (see page 3, left column, "AME/CME Preparation"):
- the human amniotic membranes matrices were collected;
- the tissues were **washed** with phosphate-buffered saline (PBS) three times to remove blood clots;
- they were then **sliced** into small pieces, frozen using liquid nitrogen and ground into fine particles using a mortar and pestle and the membrane particles were then **mixed with PBS** at a ratio of 1:1 (wt/vol) and **homogenized** on ice for 1 h;
- the membrane lysates were **centrifuged** twice at 12000 rpm at 4◦C for 10 min to obtain the supernatants;
- the supernatants were **filter** sterilized (0.22 µm pore size);
- the protein concentrations in the extracts were determined;
- the extracts were **stored** at −80˚C until use.

The process disclosed in D1 includes a centrifugation step which is excluded from the subject-matter of claim 1 of the main request by the disclaimer "without being further processed by sonication, centrifugation, or lyophilisation".

In the present case, the Board cannot identify any process step that could confer to the agent claimed in claim 13 an identifiable technical or structural difference over the product obtained in D1. The cutting step, the homogenisation step and the filtration step are indeed undefined in claim 1 of the main request, so that these steps cannot constitute distinguishing features over the process disclosed in D1 and cannot serve to characterize further the product of claim 13 obtainable by the process of claim 1.

It is also not clear why and how a process involving a centrifugation/filtration as in D1, would produce a different product from the agent of claim 13 when obtained through an undefined, but possibly very harsh or very gentle homogenisation step, followed by an undefined filtration step.

3.3.2 The appellant did furthermore not provide any evidence or convincing technical argument that the product of claim 13 can be differentiated from the product obtained in D1.

The Board could in particular not agree with the appellant's argument that claim 13, directed to an antimicrobial agent prepared by the process as defined in claim 1, is new over *inter alia* D1 for containing bioactive cells and cell fragments that are lost in the processes of the cited documents (such as D1). The crucial homogenization and filtration steps are indeed not defined in claim 1 of the main request, and there is nothing in claim 1 that excludes that the combination of homogenization and a filtration, such as a mild homogenisation and a filtration at 0.22 µm pore size, produces a homogenate which would be indistinguishable from the homogenate obtained in D1 by

centrifugation, obtention of the supernatant, i.e. elimination of the biggest particles, and filtration at 0.22 µm pore size.

Moreover, a reference to the parameter or conditions of process steps disclosed in the description or in the examples of the patent application is also irrelevant, since novelty must be assessed over the claimed subject-matter. The Board has no doubt that the selection of particular homogenization parameters disclosed on page 18 of the description of the patent application, as well as the selection of particular filtration parameters of page 20, might have an effect on the structure of the obtained antimicrobial agent, but none of these parameters are present in claim 1.

3.3.3 Consequently, claims 13 and 14 lack novelty over D1

3.4 The Board came to the same conclusion with regard to document D2.

In D2, amnion was collected under aseptic condition, **washed** with PBS containing antibiotics three times (1015 min each wash) and then immersed in SPSS containing antibiotics for 10-20 min. For amnion transplantation, the amnion and chorion were separated and the amniotic membrane was washed with PBS buffer three times, 10-15 min for each wash. A piece of tissue was **cut**, weighed and then the tissue block was placed in a homogenizer and PBS buffer was added in a 1:1 ratio by weight. The mixture was then **homogenized** by 10 strokes at 5000 rounds per minute, and each stroke lasted for 30 seconds.The homogenate was collected and placed in a centrifuge tube with PBS buffer in a ratio of 1:5. The sample was **centrifuged** at 444 g for 10 min and then the supernatant was collected. It was later

stored in liquid nitrogen (see "Material and Methods", page e316, left and middle columns).

There is no filtration step disclosed in D2 but in the absence of any specification of the filter pore size in claim 1 of the main request, this step cannot serve to differentiate the process of claim 1 or the resulting product of claim 13 from the process and final product obtained in D2.

Accordingly, claims 13 and 14 lack novelty over D2 for the same reason as explained under point 3.3 above.

3.5 The main request does not meet the requirements of Article 54 EPC.

4. Remittal to the examining division

The appellant requested a remittal to the examining division on the basis of the main request or on the basis of the auxiliary requests 1-3 for further examination, in particular with regard to Article 56 EPC.

According to Article 11 RPBA, the board shall not remit a case to the department of first instance, unless special reasons present themselves for doing so. These reasons do not exist in the present case.

The decision of the examining division was based on the assessment of the grounds of novelty and inventive step. The main reasons and documents as to this regard are included in the decision under appeal, and are the same in the appeal proceedings.

Since the primary object of the appeal proceedings is to review the decision in a judicial manner (Article 12(2) RPBA), the Board must deal with the grounds and documents on which the decision under appeal is based, including the assessment of inventive step.
Under Article 111(1) EPC, the Board therefore proceeds further with the examination of all requests with respect to Article 54 EPC and also in particular to Article 56 EPC.

5. Auxiliary request 1 - Inventive step

5.1 The claimed invention relates to a process for the preparation of human amniotic membrane homogenate that can be used as antimicrobial agent.

5.2 The examining division considered that D1 was the closest prior art, but the appellant assessed inventive step also over D9.

5.2.1 D9 discloses process steps which are similar to the process of claim 1 but with an animal amniotic membrane as starting product, and for the preparation of an amniotic membrane emulsion cross-linked facial mask. The purpose of D9 appears therefore to be more remote than the teaching of D1, which makes it less suitable than D1 as closest prior art.

5.2.2 The process disclosed in D1 discloses essentially the following steps (see also point 3.3.1 above):
- collection of the human amniotic membranes matrices;
- **washing** of the tissues with phosphate-buffered saline (PBS);
- **slicing** into small pieces, freezing in nitrogen, grinding, **mixing with PBS**, and **homogenization** on ice;
- **double centrifugation** of the membrane lysates;

- **filtration** sterilization of the supernatants (0.22 µm pore size).
- **storage** of the extracts at −80°C until use.

The only distinguishing feature of the subject-matter of claim 1 over the disclosure of D1 is the exclusion of the centrifugation step disclosed in D1 by the disclaimer "without being further processed by sonication, centrifugation, or lyophilisation" present in claim 1 of auxiliary request 1. All other steps are present in D1.

In the absence of any characterizing features, the claimed homogenisation or filtration steps cannot constitute further differences over D1. Neither can the freezing step in nitrogen or the final freezing of the obtained solution disclosed in D1 be seen as further differences, since they are not excluded by the claimed subject-matter in view of the "comprising" wording.

5.3 The appellant defines the problem as the provision of an improved process for preparing an antimicrobial agent from amniotic membrane which is time efficient and which prevents the loss of active antimicrobial molecules.

5.4 In the Board's view, there is no evidence or credible technical argumentation that could establish a technical effect over the disclosure of the prior art D1 and linked with the distinguishing feature.

The examples of the patent cannot in particular serve to show the existence of any effect. Example 1 is the only example showing the preparation of amniotic membrane homogenate in the patent application, and the process described in example 1 does not comprise any

filtration step which is one of the features of claim 1; for this reason, this example is not representative of the process of claim 1 and is not relevant. Examples 2-4 relate only to the antimicrobial activity of homogenate according to the invention, but do not disclose how the homogenates were prepared.

There is also no evidence of any time-efficiency over the process of D1 or prevention of the loss of biomaterial agent.

The Board therefore considers that the objective technical problem is the provision of an alternative process for preparing an antimicrobial agent from amniotic membrane.

5.5 With regard to obviousness, the appellant considers that the skilled person starting from D1 and seeking to solve the objective technical problem might seek to streamline the rather complex process, but would not eliminate the multiple freeze/thaw cycles, eliminate the double centrifugation as well as the sterile filtration to arrive at a process according to claim 1 of auxiliary request 1.

In the Board's view, the only relevant question with regard to obviousness, is whether the skilled person would exclude the centrifugation step from the process shown in D1. Since the problem is defined as the provision of an alternative process, the skilled person, looking for an alternative process, would consider variations of the process already known from prior art available.

D3-D5 are documents relating to the same technical field, namely the provision of amniotic membranes

having antimicrobial activity, and prepared by processes comprising some of the steps of the process claim 1, but without any centrifugation step (see D3, "Materials and Methods"; see D4, page 219, "Methods"; see D5, pages 14-15, "Methods").

A similar process without centrifugation is also described in D9 in a different context and a different purpose (see claim 6 of D9).

Accordingly, a process for the preparation of homogenates of amniotic membrane without centrifugation is known from the prior art, and is an obvious alternative.

5.6 Consequently, the subject-matter of claim 1 is not inventive and auxiliary request 1 does not meet the requirements of Article 56 EPC.

6. Auxiliary request 2 - Inventive step

6.1 In this request, the filtration step in claim 1 has been amended as follows:
"d) filtering the homogenate obtained in step c) through a mesh filter **wherein the mesh filter has a pore size from 0.5 mm to 1 mm**;".

This feature constitutes a further distinguishing feature over the process disclosed in D1, since the filtration step is performed in D1 with a filter of 0.22 µm pore size.

6.2 According to the appellant, this has the technical effect of allowing larger cell fragments, cells and small pieces of tissue to pass the filter such as to minimize loss of bioactive material. Further, the

coarser filter size allows for a quicker process with less clogging and replacement of filters.

6.3 The Board is not convinced by the appellant's arguments and concurs with the conclusion of the examining division in its decision, that no better antimicrobial effect associated with a filtration step was demonstrated. It is credible that a too small filter would remove "cells and cell fragments", but there is no evidence in the application that demonstrates that it is those cells and cell fragments which would be likely to include bioactive material and would be responsible for an antimicrobial effect. It is noted again that example 1 of the patent application does even not disclose any filtration step.

In this context, the Board notes also that the homogenisation step is still undefined in claim 1, with regard to the rotational speed or the time range, so that the claimed subject-matter encompasses also low or high intensity homogenization steps. This would have for instance as a consequence, as argued on page 18 of the description of the patent application, a diminution of the antimicrobial activity in the case of a short time range, or a damage to the antimicrobial molecules in the case of a long time range. The arguments regarding the antimicrobial activity of the appellant are therefore not relevant.

It is however clear that the use of a filter with a pore size as claimed allows for a quicker process with less clogging and replacement of filters. The objective technical problem over D1 can therefore be defined as the provision of a process for preparing an antimicrobial agent that allows for a quicker

filtration with less clogging and replacement of filters.

6.4 The solution to this problem is obvious. An immediate and obvious solution for the skilled person who intends to provide a quicker filtration process with less clogging and replacement of filters is indeed the use of a coarser mesh. A coarser mesh that has larger openings allows for higher flow rates. Accordingly, replacing the sterile filter of D1 with a known mesh filter of 0.5 - 1 mm pore size is obvious. The claimed mesh filters are known for instance from documents D12 and D14.

6.5 Consequently, the subject-matter of claim 1 is not inventive over D1 and auxiliary request 2 does not meet the requirements of Article 56 EPC.

7. Auxiliary request 3 - Inventive step

7.1 Claim 1 of auxiliary request 3 has been amended as follows:
"c) homogenizing the mixture obtained in step b) in a homogenizer **wherein the homogenizer is a homogenizer having a motor speed in the range of from 400 to 800W;** d) filtering the homogenate obtained in step c) through a mesh filter **wherein the mesh filter has a pore size from 0.5 mm to 1 mm;".**

Since D1 does not disclose the type of homogenizer used in its process, the feature regarding the homogenizer establishes an additional difference over D1, in comparison to the subject-matter of claim 1 of auxiliary request 2.

7.2 According to the appellant, the additional limitation in auxiliary request 3 of the homogenizer having a low effect motor strengthens even further the retainment of bioactives since it is a much gentler homogenization.

The examining division considered in its decision that the problem linked with the distinguishing feature of the homogenizer is a partial problem defined as the provision of a quicker homogenisation step. This partial problem is independent from the partial technical problem underlying the different filtration step.

7.3 In the Board's view, claiming the use a homogenizer having a power range of 400-800 watts does not necessarily mean that its use is gentler. Wattage indicates the power of the device, not the method of homogenization and/or the way the homogenizer is used, namely at which speed or homogenization time range.

As mentioned by the examining division in its decision, dependent claims 6 and 7 state that the homogeniser is operated at a rotational speed of from 5,000 to 24,000 rpm., 3 to 8 minutes, and may potentially turn at up to 24,000 rpm for up to 8 min. Such a homogenisation step will most likely result in complete cell disruption and the absence of cells and tissue fragments, which contradicts the existence of an effect of strengthening further the retainment of bioactive molecules in the homogenate (see description of the patent application on page 18).

In the absence of any technical specification of the homogenisation step, the use of the claimed homogenizer cannot be held responsible for any effect over the simple homogenisation on ice disclosed in D1.

The objective technical problem over D1 remains the same as for auxiliary request 2.

7.4 As mentioned for auxiliary request 2, the use of mesh filter of 0.5 - 1 mm pore size is obvious over D1.

The choice of a rotational homogeniser with a motor power of 400 - 800 W is furthermore an arbitrary choice devoid of inventive step, given that the choice has not been shown to be linked to any kind of technical effect. The use of homogenisers in the same context and with comparable speed or time range is also known from documents D2, D7 or D9:
- In D2 homogenisation takes place using a rotational homogeniser at 10 strokes at 5000 rpm, and each stroke lasted for 30 seconds (see page 316, "Material and Methods", 1st paragraph of the middle column);
- D7 employs a Tissue Tearor from Biospec Products, Inc. at speed 5 for 2 minutes for amnion homogenisation (see example 3);
- D9 uses a speed of homogenisation of more than 12,000 rpm (see claim 2).

Consequently, the claimed solution is obvious and auxiliary request 3 does not meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

C. Rodríguez Rodríguez J. Lécaillon

Decision electronically authenticated