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Datasheet for the decision of 23 January 2020

Case Number: T 2146/17 - 3.3.10

Application Number: 11192607.7

Publication Number: 2455109

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Language of the proceedings: EN

Title of invention:
Gelatin-transglutaminase hemostatic dressings and sealants

Patent Proprietor:
Lifebond Ltd.

Opponent:
GELITA AG

Headword:

Relevant legal provisions:
EPC Art. 100(a), 56, 111(1)

Keyword:
Inventive step - (no) - main request and first to fourth auxiliary requests - (yes) - fifth auxiliary request

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It can be changed at any time and without notice

Decisions cited:

Catchword:

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Case Number: T 2146/17 - 3.3.10

D E C I S I O N
of Technical Board of Appeal 3.3.10
of 23 January 2020

Appellant: (Opponent)	GELITA AG Uferstrasse 7 69412 Eberbach (DE)
Representative:	Hoeger, Stellrecht & Partner Patentanwälte mbB Uhlandstrasse 14c 70182 Stuttgart (DE)
Respondent: (Patent Proprietor)	Lifebond Ltd. Industrial Park 7 Ha-Eshel 30889 Caesarea (IL)
Representative:	Fuchs Patentanwälte Partnerschaft mbB Westhafenplatz 1 60327 Frankfurt am Main (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 18 July 2017 rejecting the opposition filed against European patent No. 2455109 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	P. Gryczka
Members:	R. Pérez Carlón
	T. Bokor

Summary of Facts and Submissions

I. The appellant (opponent) lodged an appeal against the decision of the opposition division rejecting the opposition to European patent No. 2 455 109, which had been filed on the ground of lack of inventive step (Article 100(a) EPC).

II. Claim 1 of the patent as granted, which is the main request of the respondent (patent proprietor), reads as follows:

"*A composition comprising a combination of gelatin, a denaturant and transglutaminase, wherein a ratio of an amount of said gelatin and an amount of said transglutaminase is sufficient to reduce bleeding in a wound of a mammal, wherein said gelatin has not undergone thermoreversible gelation due to said denaturant, wherein said denaturant is selected from the group consisting of urea and guanidine hydrochloride, wherein said guanidine hydrochloride is present in a concentration of between 1:2 to 2:2 of guanidine hydrochloride:gelatin, weight per weight, plus or minus 10 percent of the indicated value, and wherein said urea is present in a concentration of between 0.5:1 to 1:1 of urea:gelatin, weight per weight, plus or minus 10 percent of the indicated value, to block said thermoreversible gelation and to reduce sol-gel transition temperature of said gelatin, such that said gelatin forms a solution with transglutaminase at a temperature lower than the natural sol-gel transition temperature of standard animal gelatin, and wherein said gelatin is produced from animal origin, recombination origin or a combination thereof.*"

III. The documents filed include the following:

D1 McDermott et al., "Mechanical properties of biomimetic tissue adhesive based on the microbial transglutaminase-catalyzed crosslinking of gelatin", Biomacromolecules 2004, 5, 1270-1279

D7 Otani et al., "Effect of additives on gelation and tissue adhesion of gelatin-poly(L-glutamic acid) mixture", Biomaterials 19 (1998), 2167-2173

D10 Nomura et al., "Improvement of shark type I collagen with microbial transglutaminase in urea", Biosci. Biotechnol. Biochem, 65 (4), 982-985, 2001

D11 Yokoyama et al., "In vitro refolding process of urea-denatured microbial transglutaminase without pro-peptide sequence", Protein Expression and Purification 26 (2002), 329-335

D12 Rajagopalan et al., "Competitive inhibition of enzyme activity by urea", The Journal of Biological Chemistry, vol. 236, No. 4, 1961, 1059-1065

D13 Experimental evidence "Gelita AG, sealant burst strength test", dated 5 August 2015

D15 Kurzes Lehrbuch der Biochemie für Mediziner und Naturwissenschaftler, P. Karlson. Georg Thieme Verlag Stuttgart, 9th edition 1974, page 48

D16 Der Experimentator: Protein-biochemie, H. Rehm. Gustav Fischer Verlag, 1996, pages 94 and 95

D22 Peter et al., "Semenogelin I and semenogelin II, the major gel-forming proteins in human semen, are substrates for transglutaminase", Eur. J. Biochem., 252 (1998), 216-221

D23 US 5,508,202

D24 Schwartz et al., "The effect of fibrin-stabilizing factor on the subunit structure of

human fibrin", The journal of Clinical Investigation, vol. 50, 1971, 1506-1513

D25 Dallabrida et al., "Factor XIIIa supports microvascular endothelial cell adhesion and inhibits capillary tube formation in fibrin", Blood 2000, vol. 95, 2586-2592

D26 Greenberg et al., "Regulation of plasma factor XIII binding to fibrin in vitro" Blood, vol. 66, 1985, 1028-1034

D27 Naito et al., "Migration of cultured vascular smooth muscle cells into non-crosslinked fibrin gels", Thrombosis Research, vol. 84, 1996, 129-136

D28 Niwa et al., "Contribution of SS bonds to the elasticity of actomyosin gel in which coexisting transglutaminase was inactivated", Fisheries Science 61(3), 1995, 438-440

D29 Umakoshi et al., "Characterization of surface properties of microbial transglutaminase using aqueous two-phase partitioning method", Solvent Extraction Research and Development, Japan, vol. 15, 2008, 111-115

D30 Folk et al., "Identification of a functional cysteine essential for the activity of guinea pig liver transglutaminase", J. Biol. Chem. 1966, 241, 3238-3240

IV. The opposition division concluded that document D1 was the closest prior art. The problem underlying the claimed invention was to provide a composition that did not undergo thermoreversible gelation at application temperature while maintaining, at least to some extent, the composition's ability to reduce bleeding in a wound of a mammal by creating a barrier to fluid leakage. The claimed solution, which was characterised by containing a denaturant selected from urea and guanidine

hydrochloride, was not obvious having regard to the prior art. Document D7 disclosed urea for preventing thermoreversible gelation in the context of a chemical crosslinking, whereas claim 1 was directed to an enzymatic process. Urea was a known denaturant which would hinder transglutaminase catalysis. For that reason, the skilled person would not have considered combining these teachings and the claimed solution was inventive.

V. With its reply to the grounds of appeal, the respondent filed its first to fifth auxiliary requests.

VI. Claim 1 of the first auxiliary request contains, in addition to the features of claim 1 of the main request, the following:

"*wherein, if urea is chosen as the denaturant, the activity of transglutaminase in the gelatin-transglutaminase composition is from 25 to 400 U/g of gelatin.*"

Claim 1 of the second auxiliary request contains all the features of claim 1 of the main request, with the following added:

"*wherein the transglutaminase is microbial transglutaminase*".

Claim 1 of the third auxiliary request contains all the features of claim 1 of the main request, with the following added:

"*wherein the activity of transglutaminase in the gelatin-transglutaminase composition is from 25 to 400 U/g of gelatin*".

Claim 1 of the fourth auxiliary request limits the activity of transglutaminase with respect to claim 1 of the third auxiliary request to: "*from 40 to 200 U/g of gelatin*".

Lastly, claim 1 of the fifth auxiliary request restricts the composition of claim 1 of the main request by requiring the denaturant to be guanidine hydrochloride.

VII. The arguments of the appellant relevant to the present decision were as follows.

Document D1 was the closest prior art and disclosed all the features of claim 1 of the main request, except the required denaturant. The claimed composition only solved the the problem of avoiding thermoreversible gelation at application. Even if the problem of providing a composition suitable for reducing bleeding in a wound of a mammal were also considered as solved, the claimed solution, characterised by a defined amount of a denaturant selected from guanidine hydrochloride and urea, was obvious having regard to D7. For this reason, the compositions of claim 1 of the main request and of the second auxiliary request were not inventive.

The skilled person would not find any problem in optimising the relative amount of transglutaminase and gelatin in order to reduce bleeding in a wound of a mammal. The compositions of claim 1 of each of the first, third and fourth auxiliary requests were thus not inventive.

The composition of claim 1 of the fifth auxiliary request was not inventive for the same reasons as that of claim 1 of the main request, since guanidine

hydrochloride was a well-known denaturant, equivalent to urea (D12, D15).

VIII. The respondent agreed that document D1 was the closest prior art. The problem underlying the claimed invention was to provide a composition suitable for reducing bleeding in a wound of a mammal which did not undergo thermoreversible gelation at application. The solution to that problem was the composition of claim 1, characterised by a defined amount of a denaturant selected from guanidine hydrochloride and urea. The skilled person would not have sought the solution in D7, as it related to chemical crosslinking and not to an enzymatic reaction. In addition, urea was a known denaturant of proteins (D10, D11, D22-D30) which would likely hinder the catalytic activity of transglutaminase. For this reason alone, claim 1 of the patent in suit and of each of the first to fourth auxiliary requests was inventive.

The skilled person would not be prompted to modify the relative amount of transglutaminase to gelatin disclosed in D1, as this document achieved excellent results. For that reason also, the compositions of claim 1 of each of the first, third and fourth auxiliary requests were inventive.

Claim 1 of the fifth auxiliary request required the presence of guanidine hydrochloride as denaturant. As there was no evidence on file showing that this compound could have any influence on the thermoreversible gelation of gelatin, the claimed composition was inventive.

IX. Oral proceedings before the board of appeal took place on 23 January 2020.

X. The final requests of the parties were as follows:

- The appellant requested that the decision under appeal be set aside and that European patent No. 2 455 109 be revoked.

- The respondent requested that the appeal be dismissed (main request), i.e. that the patent be maintained as granted and the rejection of the opposition be confirmed, or that the patent be maintained on the basis of one of the first to fifth auxiliary requests filed with the reply to the grounds of appeal dated 11 June 2018. It also requested that documents D35 and D36 not be admitted.

XI. At the end of the oral proceedings, the decision was announced.

Reasons for the Decision

1. The appeal is admissible.

Inventive step - main request

2. Claim 1 of the patent as granted is directed to a composition comprising a combination of gelatin, a denaturant selected from urea and guanidine hydrochloride, and transglutaminase. Claim 1 further requires a defined relative amount of denaturant with respect to gelatin, and the ratio of gelatin to transglutaminase to be sufficient to reduce bleeding in

a wound of a mammal.

3. Closest prior art

The parties agreed with the opposition division that document D1 is the closest prior art. The board sees no reason to differ.

It was common ground that document D1 discloses a soft tissue sealant composition comprising gelatin and transglutaminase, differing from the composition of claim 1 in that it lacks a denaturant.

4. Technical problem underlying the invention

The respondent argued that the technical problem underlying the claimed invention was to provide a composition suitable for reducing bleeding in a wound of a mammal which did not undergo thermoreversible gelation at application.

5. Solution

The claimed solution is the composition of claim 1, characterised in that it contains a denaturant selected from urea and guanidine hydrochloride, in defined relative amounts with respect to gelatin.

6. Success

In the following, it will be examined whether the subject-matter of claim 1 is inventive, assuming that the technical problem as defined by the respondent has been credibly solved by the features of this claim. Since the conclusion of the board on inventive step is negative for the reasons below, it is not necessary to

further examine whether the problem has been solved.

7. It remains to be examined whether the proposed solution would have been obvious for the skilled person in view of the prior art, in essence whether it was obvious for the skilled person to add to the composition a denaturant as specified in the claim in order to avoid thermoreversible gelation of gelatin at application.

7.1 D7 discloses gelation and tissue adhesion of mixtures of gelatin and poly(L-glutamic acid). This document relates to the problem of thermoreversible gelation of gelatin at room temperature ("spontaneous physical gelation", page 2167, right column, lines 11-16, Figure 1), and discloses the effect of a number of additives, among which urea was the most promising (Table 1).

D7 also discloses that a 1:1 urea:gelatin mixture by weight (135 mg/mL of gelatin, see point 2.2 of D7, and 135 mg/mL of urea) did not undergo spontaneous gelation (Table 1; Table 2, page 2169, right column, penultimate sentence), and that 67.5 mg/mL urea retarded gelation of a 135 mg/mL gelatin solution (0.5:1 urea:gelatin mixture by weight) by a factor of five over mixtures without any additive (Table 2, second entry).

D7 further discloses that urea induces only a small change in the bonding strength of the final crosslinked gels (Figure 4; page 2170, point 3.3).

7.2 The skilled person trying to avoid thermoreversible gelation of gelatin at room temperature thus finds in D7 the teaching that urea is an additive suitable for that purpose, that it had been successfully used in the field of tissue adhesives, and that it prevents thermoreversible gelation of gelatin at relative

amounts which fall within those required by claim 1. The skilled person would apply this teaching to the soft tissue adhesive of D1 and would thus arrive at the claimed subject-matter without using inventive skills.

7.3 For these reasons, the board concludes that the claimed composition is not inventive (Article 56 EPC).

7.4 The respondent argued that document D7 relied on carbodiimide chemistry (Figure 1; 2.2. Materials) and not on enzymatic crosslinking, as in D1. For this reason alone, the skilled person would not have combined the teaching of documents D1 and D7.

However, thermoreversible crosslinking is a process inherent to gelatin which precedes irreversible crosslinking, be it chemical or enzymatic. The skilled person would not consider the effect of urea to be limited to the system of D7, but to be generally applicable.

This argument is thus not convincing.

7.5 The respondent further argued that urea was a known protein denaturant (D12, D16). D12 disclosed the inhibition of enzymes by urea to be a general phenomenon. The skilled person would thus have expected it to render transglutaminase inactive and would therefore consider its use to be incompatible with the sealant of D1.

The board agrees with the respondent that urea is known to be a denaturant. However, the passage of D12 bridging left and right columns on page 1063 on which the respondent relies discloses urea inhibition to be "a general phenomenon extending to many enzymes not

investigated in this study". This sentence is far from disclosing that (almost) every enzyme is inhibited by urea, as the respondent argued. In fact, D12 explicitly discloses that not every enzyme is inhibited by urea (page 1061, left column, last paragraph; page 1059, left column, lines 17-19).

This argument is thus not convincing.

7.6 The respondent argued that the adhesives of D1 would have needed 2.8 to 4.4 M urea in order to arrive at the required 1:1 mixture by weight of urea and gelatin. Documents D10, D11 and D22 to D30 showed the inactivation of various transglutaminases under such conditions. For these reasons also, the skilled person would not have added urea to the compositions of D1.

Document D10 relates to the inhibition of fibril reconstruction by urea (Figures 1 and 2), which is in fact reverted by microbial transglutaminase, showing, contrary to the appellant's argument, that microbial transglutaminase is not inhibited by urea in the conditions of D10.

Document D11 discloses the denaturalisation of microbial transglutaminase by 8 M urea, which is a higher concentration than would have been required by applying the teaching of D7 to that of D1. Contrary to the respondent's argument, page 333, left column, paragraph 1 of D11, does not disclose that 0.4 M urea inhibits transglutaminase, but relates to the effect of pH on its refolding. Similarly, documents D28, D29 and D30 disclose denaturing transglutaminase with 8 M urea.

Document D22 relates to the crosslinking of semenogelins (gel-forming proteins) with factor XIIIa,

which is a transglutaminase. On page 218, D22 discloses that 2 M urea rendered crosslinking markedly slower, but attributes the effect to semenogelins becoming less suitable substrates, not to transglutaminase inhibition.

Table 2 of document D23 discloses coagulation times of normal plasma and factor XIII-deficient plasma in the presence of 5 M urea. Comparison of these data shows that factor XIII retained activity in the presence of 5 M urea, as it decreased coagulation times with respect to plasma lacking transglutaminase.

Document D24 discloses that factor XIII can be removed from fibrinogen by treatment with 3.3 M urea followed by dialysis. The authors speculate that it could have been selectively and irreversibly denatured, but they did not further investigate either the fate of factor XIII or the effect of dialysis. Documents D25 and D26 merely refer back to D24. D27 discloses factor XIII inactivation by the same process as D24.

To sum up, the evidence provided by the respondent shows that 8 M urea deactivates transglutaminases, and that the effect is markedly lower at lower concentrations. This is in fact also the conclusion of the patent in suit, see paragraph [0326].

The skilled person would have been aware that transglutaminase activity could have been lower in the presence of urea, but would nevertheless have combined the teaching of D1 and D7 in order to provide a composition containing gelatin which could be handled at room temperature without showing thermoreversible crosslinking. The board agrees with the respondent's argument that the skilled person would have had some

doubts as to whether urea could have been successfully used, but this does not preclude that the skilled person would nevertheless have tried to use it.

7.7 The respondent also argued that the mixtures of D7 contained a second polymer, which played an essential role in the tissue adhesive of D7 and was absent from the compositions of D1. For this reason also, the skilled person would not have combined the teaching of documents D1 and D7.

However, D7 discloses that urea exclusively affected gelatin, not the second polymer of the adhesive. This argument is thus not convincing either.

7.8 The respondent argued that document D7 disclosed gels obtained from gelatin B, whereas those of D1 were obtained from gelatin A. This was a further difference between these documents which would discourage the skilled person from combining their teaching.

However, it was not shown that the problem of thermoreversible crosslinking of gelatin at room temperature is dependent on the type of gelatin employed. The skilled person would thus have considered the solution to that problem disclosed in D7 to be generally applicable.

7.9 The respondent argued that Figure 4 of D7 disclosed decreasing hydrogel bonding strength with increasing amounts of urea. This would have also discouraged the skilled person from considering its teaching in the context of tissue sealants.

However, Figure 4 shows only a small reduction in the strength of the gel obtained with a 1:1 gelatin:urea

mixture. The accompanying text on page 2170 (point 3.3) discloses that "when urea was added, the bonding strength did not greatly decrease", and the first paragraph on the following page discloses that "there was no significant difference in the adhesion strength between the hydrogels with and without urea".

Therefore, this argument is also unconvincing.

7.10 As the claimed composition is not inventive (Article 56 EPC), the opposition ground pursuant to Article 100(a) EPC precludes the maintenance of the patent as granted.

Inventive step - first to fourth auxiliary requests

8. Claim 1 of the first auxiliary request requires a relative amount of transglutaminase from 25 to 200 U/g of gelatin, which is higher than disclosed in document D1 (15 U/g gelatin).

In the context of claim 1 of the main request, which requires a ratio of gelatin to transglutaminase "sufficient to reduce bleeding", the respondent explained that the skilled person would have found no difficulty in adjusting the relative amount of transglutaminase and gelatin in order to efficiently reduce bleeding.

For the same reason, the board concludes that optimising the relative amount of transglutaminase to gelatin in the sealant compositions of D1 falls within the normal skills of the person of the art. The composition of claim 1 of the first auxiliary request is thus not inventive (Article 56 EPC).

9. The respondent argued that document D1 disclosed that the gel time achieved with compositions having 15 U of transglutaminase per gram of gelatin was convenient (page 1277, Conclusions), and that the tissue adhesives obtained from those compositions penetrated and interlocked with tissue (page 1274, left column, lines 23-28). It concluded that the skilled person had no reason to modify the excellent sealants of D1.

However, D1 discloses that the properties of the adhesive can be controlled by varying the enzyme activity (page 1274, left column, lines 29 and 30). It could be expected that the presence of a denaturant such as urea would affect the final properties of the adhesive obtained. The skilled person would have been aware of the need for adjusting the relative amounts of the components of the composition, and in particular of increasing the activity of the crosslinking catalyst. This argument is thus not convincing.

10. Claim 1 of the second auxiliary request requires that the transglutaminase is a microbial transglutaminase. As the closest prior-art document D1 already discloses a composition containing microbial transglutaminase, this feature does not add any further difference; the finding on the issue of inventive step thus remains the same as for claim 1 of the main request.

11. Claim 1 of the third auxiliary request contains the same features as claim 1 of the first auxiliary request as far as the embodiment requiring urea is concerned. For this reason, the arguments presented for the latter also hold for the third auxiliary request.

12. Claim 1 of the fourth auxiliary request further limits the relative amount of transglutaminase by raising its

lowest limit to 40 U/g of gelatin. As explained above in point 8., the skilled person would optimise the parameters of the composition of D1 and thus would have arrived at the required values without using inventive skills.

13. For these reasons, the first to fourth auxiliary requests are not inventive (Article 56 EPC) and thus not allowable.

Fifth auxiliary request

14. Claim 1 of the fifth auxiliary request is directed to the embodiment of claim 1 of the patent as granted, according to which the denaturant is guanidine hydrochloride.

15. The appellant argued that the problem underlying the claimed invention was merely to provide a composition comprising transglutaminase and gelatin which did not undergo thermoreversible gelation at application.

16. The claimed solution is characterised by the use of guanidine hydrochloride. It was common ground that, having regard to the results put forward in Example 1 of the patent in suit, paragraphs [0250] ff, the problem of avoiding thermoreversible gelation is credibly solved by the claimed composition.

17. The appellant argued that guanidine hydrochloride was known to be a denaturant equivalent to urea (D12, D15) and, for that reason, the reasoning with respect to the latter should apply analogously.

However, there is no evidence on file showing that guanidine hydrochloride could prevent gelatin

thermoreversible gelation, let alone in the context of tissue adhesives. Document D12 discloses guanidine hydrochloride only as an enzyme inhibitor. Document D15 discloses urea and guanidine in the context of denaturing proteins, not peptides, let alone gelatin.

The board has concluded that the skilled reader would not have expected urea to denature every protein. For the same reason, it cannot be concluded that every known denaturant, such as guanidine hydrochloride, may act on gelatin. Lacking disclosure on the specific effect of guanidine hydrochloride on gelatin, the skilled person would not have considered applying it to the compositions of D1 in order to avoid thermoreversible gelation.

For this reason, the compositions of claim 1 of the fifth auxiliary request and of dependent claims 2 to 15 involve an inventive step. By the same token, the haemostatic dressing or bandage of claim 16 and the medical device of claim 17 containing those compositions are also inventive within the meaning of Article 56 EPC.

18. The appellant did not raise any further objections with respect to the fifth auxiliary request, nor are any other objections apparent to the board.

Procedural matters

19. As documents D35 and D36 are not relevant for this decision, there is no need to decide on their admission into the proceedings.

Remittal

20. The description of the patent as granted contains subject-matter not encompassed by the claims of the fifth auxiliary request (see Example II) and thus requires amendment (Article 84 EPC). The board decided to make use of its discretion to remit the case to the opposition division for the description to be adapted (Article 111(1) EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent with claims 1 to 17 of the fifth auxiliary request filed with the response to the grounds of appeal dated 11 June 2018 and a description to be adapted thereto.

The Registrar: The Chair:

C. Rodríguez Rodríguez P. Gryczka

Decision electronically authenticated