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Datasheet for the decision of 15 October 2008

Case Number: T 1172/06 - 3.3.04

Application Number: 95201872.9

Publication Number: 0714665

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Language of the proceedings: EN

Title of invention:
Osteogenic devices

Patentee:
STRYKER CORPORATION

Opponent:
Biopharm Gesellschaft zur biotechnologischen Entwicklung von Pharmaka mbH

Headword:
Osteogenic proteins/STRYKER

Relevant legal provisions:
EPC Art. 54, 56, 87

Keyword:
"Novelty, inventive step (yes)"

Decisions cited:
G 0001/92, G 0002/98, T 0666/89, T 0823/96, T 0604/04, T 1329/04, T 0665/05, T 1599/06

Catchword:
see point 9.2

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1172/06 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 15 October 2008

Appellant (Opponent)	Biopharm Gesellschaft zur biotechnologischen Entwicklung von Pharmaka mbH Czernyring 22 D-69115 Heidelberg (DE)
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Respondent: (Patent Proprietor)	STRYKER CORPORATION 2725 Fairfield Road P.O. Box 4085 Kalamazoo Michigan 49003-4085 (US)
Representative:	Price, Vincent Andrew Fry Heath & Spence LLP The Gables Massetts Road Horley Surrey RH6 7DQ (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 30 May 2006 rejecting the opposition filed against European patent No. 0714665 pursuant to Article 102(2) EPC 1973.**

Composition of the Board:

Chairman: M. Wieser
Members: G. Alt
F. Blumer

Summary of Facts and Submissions

I. This is an appeal by opponent 01 (appellant) against the opposition division's decision to reject the opposition against the European patent No. 0 714 665 with the title "Osteogenic devices" pursuant to Article 102(2) EPC 1973.

II. The opposition was based on Article 100(a) EPC on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC).

III. The opposition division decided that claims 1 to 9 as granted fulfilled the requirements of the EPC.

Independent claims 1 and 5 as granted read:

"1. A process for the production of active osteogenic protein in unglycosylated form, the protein comprising a TGF-beta-like domain containing seven cysteines, the process comprising the steps of:

(a) providing genetic material encoding the osteogenic protein,
(b) introducing the genetic material into a prokaryotic host cell;
(c) expressing the genetic material in the prokaryotic host cell, and
(d) refolding the expressed protein by oxidation to produce an active osteogenic protein comprising a pair of polypeptide chains bonded in the unreduced state to from a homo- or heterodimeric species having a conformation such that the pair of polypeptide chains

is capable of inducing endochondral bone formation when disposed within a matrix and implanted in a mammal.

5. A process for producing an osteogenic device comprising disposing the protein of any one of the preceding claims in a matrix."

IV. The opposition division decided that document D1 (see section IX below) did not destroy the novelty of the subject-matter of claim 1 as granted. It further held that the process according to claim 1 as granted was not entitled to the first and the second priority date and that therefore documents E1, E2 and D7 (see section IX below) were state of the art. Finally, the opposition division considered that, starting from document D1 as the closest prior art document, the problem to be solved was the provision of recombinantly produced unglycosylated proteins capable of inducing bone formation. However, in view of the particular difficulties related to obtaining fully osteogenic proteins (such difficulties being disclosed in documents E1, E2 and D7), the skilled person could only hope to succeed but had no reasonable expectation of success in obtaining such proteins. Therefore, the opposition division acknowledged an inventive step.

V. In response to the appellant's grounds for appeal, the respondent (patent proprietor) filed a submission to which a copy of document E5 was annexed.

VI. The board sent a communication in which it inter alia informed the parties that the issue of whether or not the disclosure in the patent made it plausible that the solution as claimed had actually been achieved, which

according to the case law of the boards of appeal is a criterion to be determined in the context of the examination as to inventive step, had not yet been considered. The board noted that the patent disclosed only one example of the prokaryotic production of an active osteogenic protein inducing cartilage and bone formation. Moreover the board noted that document E1 was apparently published after the third priority date, that the respondent had however submitted during opposition proceedings that the paper had been presented at a congress that took place before that date and that this seemed to be accepted by the appellant.

VII. The respondent informed the board in reply to its communication that it did not intend to be represented at the oral proceedings.

VIII. Oral proceedings took place on 15 October 2008 in the absence of the respondent.

The appellant requested that the decision under appeal be set aside and that European patent No. 0714 665 be revoked.

The respondent had requested in writing that the appeal be dismissed or that the decision under appeal be set aside and the patent be maintained on the basis of the claims of either of four auxiliary requests.

The respondent had moreover requested that, in the event that the board considered the claims to have an earlier priority date than 23 February 1989, the claims

to priority based on documents US179406 and US232630 be abandoned.

At the end of the proceedings the board announced its decision.

IX. The following documents are mentioned in the present decision:

D1: WO 88/00205

D3: EP-B1-0 114 506

D4: J.Biol.Chem., vol. 261, No. 29, October 1986, pages 13838-13844, Winkler, M.E. et al.

D5: EMBO Journal, vol. 4, no. 3, 1985, pages 775-780, Schoemaker J.M. et al.

D6: WO 86/05809

D7: Proc.Natl.Acad.Sci., vol. 85, December 1988, pages 9484-9488, Wang, E.A. et al.

D10: US 4,563,350

E1: Connective Tissue Research, vol. 20, 1989, pages 313-319, Rosen, V. et al.

E2: Science, vol. 242, December 1988, pages 1528-1534, Wozney, J.M. et al.

E5: Biochem. J., vol. 240, 1986, pages 1-12, Marston, F.A.O.

"Nr. 14": EP-A-0 222 491

X. The appellant's submissions in writing and during oral proceedings, in so far as relevant to the present decision, may be summarised as follows:

Novelty

Document D1 explicitly disclosed all the features of claim 1 except the feature in point (d) that the expressed protein is refolded by oxidation. This feature was however implicitly disclosed because the skilled person knew that, after expression in prokaryotic host cells, in the majority of cases and in particular in the case of proteins containing a high number of cysteines the expressed proteins were located in an incorrectly folded and thus inactive form in inclusion bodies, and that one of the measures for releasing them therefrom in active form was refolding by oxidation (documents D3 to D6).

Entitlement to priority

The first priority document, US 179406 filed on 8 April 1988, and the second priority document, US 232630 filed on 15 August 1988, disclosed all the elements of the process according to claim 1 except that it was for the production of proteins comprising "a TGF-beta-like domain containing seven cysteines". However, the patent itself provided evidence that the process disclosed in the priority document resulted in such proteins. Therefore, the invention as formulated in claim 1 was the same as that disclosed in the first

and second priority documents. Consequently, claim 1 was entitled to the first and second priority date and none of documents E1, E2 or D7 was prior art.

Inventive step

Document E2 disclosed inter alia the proteins BMP-2A and BMP-3, that they had a TGF-beta-like domain containing seven cysteine residues and that, after their prokaryotic expression, none of them had either cartilage or bone forming activity. Therefore, document E2 was the closest prior art document.

The problem to be solved was the provision of a process for the prokaryotic recombinant production of osteogenic proteins which had a TGF-beta-like domain containing seven cysteine residues and were active, i.e. capable of inducing bone formation.

This problem was solved by including the step of "refolding the expressed protein by oxidation" at the end of the prokaryotic expression process.

The patent only disclosed one single set of parameters for carrying out the process, namely that used during the production of active OP-1 protein. The process according to claim 1 related to the production of proteins characterised only in that they comprised a TGF-beta-like domain containing seven cysteine residues. It was common general knowledge that the combination of the primary structure and the unfolding/refolding conditions was decisive for obtaining a correctly folded and thus active protein. Therefore, the restricted disclosure in the patent did

not make it plausible that the subject-matter of claim 1 solved the problem. Consequently, an inventive step should not be acknowledged.

An inventive step should also be denied because the subject-matter of claim 1 was obvious. The skilled person would not be deterred by the disclosure in documents E1, E2 and D7 from attempting to generate active osteogenic proteins in prokaryotic cells. Instead, the skilled person would have considered it evident that optimisation of the prokaryotic production process was alone necessary and he or she knew in view of documents D3 to D6 which measures had to be taken. It was reported in these documents that the expression of heterogolous proteins in prokaryotic cells resulted in inclusion bodies which contained the proteins in an aggregated, inactive form and from which they had to be solubilised and then refolded into the correct form by, for example, oxidation.

XI. The respondent's submissions in writing, in so far as relevant to the present decision, may be summarised as follows:

Novelty

The mere mention of prokaryotic expression in document D1 did not amount to an implicit disclosure of either inclusion bodies or a refolding step because neither of these was an inevitable consequence of prokaryotic expression. A wide range of proteins, including those having disulphide bonds, was expressed in Escherichia coli (E.coli) in soluble, active form (document E5).

Entitlement to priority

The feature "a TGF beta-like domain containing seven cysteines" and the step of "refolding the expressed protein by oxidation" were not disclosed in either the first or the second priority document. Therefore, claim 1 was only entitled to the third priority date, i.e. 23 February 1989. Consequently, documents E1, E2 and D7 were prior art.

Inventive step

Document D1, the closest prior art document, when read together with documents E1, E2 and D7, would not have provided any incentive to produce unglycosylated proteins comprising a TGF-beta-like domain with seven cysteines because they all reported failure to isolate such proteins in an active form after recombinant expression. Thus, it was not obvious to try to obtain such proteins after prokaryotic expression, nor was there a reasonable expectation of success. Therefore, an inventive step was to be acknowledged.

Reasons for the decision

Admission of document E5

1. Document E5 was filed with the respondent's response to the appellant's statement of the grounds for appeal as evidence of the common general knowledge in the field of prokaryotic expression of eukaryotic proteins. Since some of the parties' arguments hinge on this aspect, the document is, in the board's view, relevant to

arriving at a decision. Moreover, the document was filed at the earliest point in time during the appeal proceedings and the appellant did not object to its introduction. Hence, the board has decided to admit the document into the proceedings.

Novelty

2. A prior art document is only considered as anticipating claimed subject-matter if all the features used for its characterisation in the claim are clearly and unambiguously derivable from that document, either explicitly or implicitly.

2.1 The feature "refolding the expressed protein by oxidation" in part (d) of claim 1 is not explicitly disclosed in document D1 and this is not disputed by the respondent, who maintains, however, that the feature in question is implicitly derivable from that document. In the appellant's view, the disclosure in document D1 that the osteogenic proteins may inter alia be expressed in bacterial cells would clearly and unambiguously imply to the skilled person that such proteins would be sequestered in inclusion bodies from which they would then have to be liberated by oxidation.

2.2 The term "implicit disclosure" relates to matter which is not explicitly mentioned in a document, but which is a clear and unambiguous consequence of what is explicitly mentioned and which therefore forms part of the disclosure content of this document. Common general knowledge is taken into account in deciding what is clearly and unambiguously implied by explicit

disclosure in a document (for example, decision T 823/96 of 28 January 1997, point 4.5 of the reasons).

2.3 Document E5 is a review article published in 1986 and relating to the purification of eukaryotic polypeptides synthesised in Escherichia coli (E.coli). The information contained therein may therefore be considered to reflect the common general knowledge in the field of expression of eukaryotic proteins in prokaryotic cells at the priority date of the patent (see point 9.3 below).

2.4 The document discloses that foreign proteins expressed in E.coli may be found in the bacterial cytoplasm in either soluble or insoluble form (page 1, sentence bridging the two columns, and page 8, middle of the first column). This applies likewise to proteins with cysteine residues (see, for example, Table 1 and the observations in point 14.8 below). The insoluble proteins often form aggregates, the so-called inclusion or refractile bodies (page 1, second column; Table 1, where "supernatant" denotes that the protein is soluble and "pellet" that it is insoluble in the form of inclusion bodies). The formation of inclusion bodies may result from intra- or intermolecular non-covalent interactions, for example ionic bonds (page 6, second column, first sentence, of first full paragraph) or from intra- or intermolecular covalent interactions, for example disulphide bonds (page 7, first column, first full paragraph). Owing to these interactions the proteins contained in inclusion bodies may take on a non-natural conformation which precludes their activity.

2.5 If soluble active proteins are to be recovered from inclusion bodies it is necessary, first, to solubilise them by using denaturing agents, for example guanidinium chloride (e.g. page 6, second column, last paragraph, of document E5), in order to disrupt any unwanted or incorrect bonds. This step will also cause the proteins to unfold. Then the conditions have to be adjusted in such a way that the solubilised, unfolded proteins refold into the correct three-dimensional formation.

2.6 In view of this common general knowledge, the skilled person would therefore, in the board's view, implicitly derive from the explicit disclosure in document D1 of the possibility of prokaryotic expression of osteogenic proteins the information that these proteins may either be sequestered in inclusion bodies or may be soluble or both. However, since the formation of inclusion bodies is not the only consequence of prokaryotic expression of osteogenic proteins, no clear and unambiguous implicit disclosure of such an event is derivable from document D1. Consequently (see point 2 above), this feature cannot be considered to be disclosed in document D1.

2.7 It follows from the observations in points 2.4 and 2.5 above that the formation of inclusion bodies is a prerequisite for the step consisting of "refolding by oxidation". Therefore, if the occurrence of inclusion bodies after prokaryotic expression cannot be considered as disclosed in document D1 (point 2.6 above), this also holds true a fortiori for the process step of "refolding the expressed protein by oxidation".

2.8 Hence, since at least feature (d) in claim 1 is not disclosed in document D1, the novelty of claim 1 and its dependent claims 2 to 4 vis-à-vis document D1 is acknowledged.

2.9 The respondent has not raised novelty objections on the basis of other documents on file with regard to the subject-matter of claims 1 to 4. The board also sees no reason for doing so.

3. Claim 5 is the second independent claim among the claims as granted. It relates to a process for producing an osteogenic device which involves disposing the protein according to any one of the preceding claims in a matrix.

3.1 However, the preceding claims 1 to 4 do not relate directly to a "protein", but rather to a process for producing a protein (section III above). Therefore, in the board's view, claim 5 must be construed as relating to a process for producing an osteogenic device, comprising as one of its features the process referred to in any of claims 1 to 4 resulting in a protein which is to be disposed in the matrix.

3.2 The appellant has not raised an objection of lack of novelty against the subject-matter of claim 5 and the board sees no reason to do so, particularly in view of document D1 for the reasons given above in points 2 and 3. Thus, the subject-matter of claim 5 and its dependent claims 6 to 9 is considered novel.

4. The requirements of Article 54 EPC are fulfilled.

Entitlement to priority

5. Pursuant to Article 87 EPC, the right of priority from an earlier application for subject-matter claimed in a later European application can only be acknowledged "in respect of the same invention".

The Enlarged Board of Appeal in decision G 2/98 (OJ EPO 2001, 413) held that identity of invention exists if the skilled person derives the subject-matter of a claim, i.e. the features by which the invention is characterised in the claim, directly and unambiguously, be it explicitly or implicitly, by taking into account the common general knowledge, from the application document whose priority is claimed as a whole (points 2, 4 and 9 of the reasons).

6. The appellant submits that the first priority document, US 179406 filed on 8 April 1988, and the second priority document, US 232630 filed on 15 August 1988, implicitly disclose the feature that the process disclosed in these documents is for the production of proteins comprising "a TGF beta-like domain containing seven cysteines" because proteins comprising "a TGF beta-like domain containing seven cysteines" are the inevitable result when that process is carried out.

7. The board understands the appellant's argument to rely on case law developed in the context of the evaluation of novelty, whereby

(i) a product which is the inevitable, though undisclosed, result of carrying out a process described in a document is considered as being implicitly made

available by the disclosure in that document (T 666/89; OJ EPO 1993, 495), and

(ii) properties of a product which are not disclosed, but which are intrinsic to the product, are considered as being implicitly made available by the disclosure of that product (G 1/92; OJ EPO 1993, 277).

7.1 Even if these principles are regarded as applicable for the purposes of determining the disclosure content of priority documents, the board cannot, for the following reasons, conclude that the feature "a TGF beta-like domain containing seven cysteines" is implicitly derivable from the disclosure in these documents.

Inevitable result

8. Neither of the first and second priority documents discloses any full-length sequence of an osteogenic protein, but only short amino acid fragments derived from such a protein (page 14 in both documents). Hence, in view of these documents, the first step to be taken in the process for the production of osteogenic proteins comprising "a TGF beta-like domain containing seven cysteines" is a screening procedure with the disclosed fragments as probes in order to provide genetic material encoding a full-length osteogenic protein. Parameters of screening procedures such as the type of library to be screened, the type of probe or the hybridisation conditions are decisive for the nature of the nucleic acid which will be retrieved. With the exception of the disclosure of the specific amino acid fragments which could be used as probes, none of these conditions is disclosed in either the

first or the second priority document. Therefore, the board is not convinced that proteins which all share the structural element of "a TGF beta-like domain containing seven cysteines" would be the inevitable result of, and therefore implicitly disclosed by, the disclosure of the process in the first and second priority documents.

Intrinsic features

9. Even if such proteins were inevitably obtained by the process disclosed in the first and second priority documents, and assuming that the provision of the protein made the amino acid sequence of such a protein available, the question arises whether or not the feature "a TGF beta-like domain containing seven cysteines" would be made available by the disclosure of this sequence.

9.1 It has been held by the Enlarged Board of Appeal in the context of the question of the novelty of a commercially available product that nothing which goes beyond its composition or internal structure is implicitly disclosed by virtue of its availability to the public (decision G 1/92, supra; point 3 of the reasons). In other words, only intrinsic, not extrinsic, features of a product are considered as being disclosed by the disclosure of the product itself.

With regard to extrinsic features the Enlarged Board stated:

"Extrinsic characteristics, which are only revealed when the product is exposed to interaction with

specifically chosen outside conditions, e.g., reactants or the like, in order to provide a particular effect or result or to discover potential results or capabilities, therefore point beyond the product per se as they are dependent on deliberate choices being made (point 3 of the reasons)."

9.2 A TGF-beta-like domain is a structural motif embedded within a longer protein. In such a domain the characteristic seven cysteine residues are scattered over a stretch of more than 100 amino acid residues at defined distances (see Figure 6 of document E2). In the board's view, this pattern is not striking if the amino acid sequence of the whole protein is considered, in contrast to, for example, an uninterrupted stretch of seven cysteine residues. The motif only becomes visible when the protein sequence is aligned with the sequence of the "correct" counterpart, i.e. a protein with a TGF-beta-like domain. The board therefore considers that, although a TGF-beta-like domain is an internal structural element of a protein, it can nevertheless not be regarded as an intrinsic feature of that protein. Consequently, it cannot be considered as made available by the disclosure of the product itself.

9.3 Thus, the board concludes that the feature that the process is for the production of proteins comprising "a TGF beta-like domain containing seven cysteines" is implicitly disclosed in neither the first nor the second priority document. Hence, the process as claimed in claim 1 and that disclosed in the first and second priority documents do not relate to the same invention. Therefore, the subject-matter of claim 1 is not

entitled to the priority dates of 8 April 1988 and 15 August 1988.

9.4 Documents E2 and D7, both published in December 1988 and thus before the filing of the third priority document on 23 February 1989, are therefore comprised in the state of the art pursuant to Articles 54(2) and 89 EPC.

9.4.1 The publication date indicated on document E1 is "1989". The respondent during opposition proceedings submitted that the paper had originally been presented at a congress held in October 1988. On the basis of the parties' submissions in this respect (sections VI and VII above) the board cannot come to a final decision on what information was made available at the congress. However, no decision has to be taken on this point, because, in the board's view, the document is not relevant to the issue to be decided (see point 14.4 below).

Inventive step

Closest prior art and problem to be solved

10. The appellant regarded document E2 and the respondent document D1 as the closest prior art document.

Document D1 discloses the nucleic and amino acid sequences of human osteogenic proteins hBMP-1, hBMP-2 class I and class II (corresponding to BMP2A and BMP2B disclosed in document E2 (see below) and to BMP2a and BMP2 of the patent in suit) and hBMP-3, and suggests that the nucleic acid could be inserted into

prokaryotic and eukaryotic hosts for recombinant production of active osteogenic proteins (pages 50 to 52). In a worked example, the expression of hBMP-1, which has a domain homologous to a domain in epidermal growth factor (EGF) (see page 33 of document D1) in the eukaryotic COS cells, is disclosed. The protein thus produced is able to induce cartilage formation (page 55). Recombinant expression of BMP-2 is not reported. It is however stated on page 55 that "[f]urther, in a rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic (note by the board: i.e. cartilage-inducing) activity."

Document E2, which inter alia cites as authors the three named inventors of document D1, also discloses the nucleic acid and amino acid sequences of BMP-1, BMP-2A, BMP-2B and BMP-3. It is further disclosed that, unlike BMP-1 which has an EGF-like domain (page 1533, first column), the proteins BMP-2A, BMP-2B and BMP-3 have a TGF-beta-like domain with seven cysteine residues (Figure 6A; page 1531, last paragraph, second column; page 1533, first column). Furthermore, the recombinant expression in prokaryotic and eukaryotic systems of BMP-1, BMP-2A and BMP-3 is reported. All of the three proteins induced cartilage formation after expression in eukaryotic cells. After expression in the prokaryotic host E. coli, cartilage-inducing activity was reported for BMP-1, but no activity at all was detected in the case of BMP-2A and BMP-3 (page 1531, second column; see also point 14.2 below).

10.1 Since, in contrast to document D1, document E2 discloses the actual prokaryotic expression of proteins having a TGF-beta-like domain containing seven cysteine

residues and the results of such an expression in terms of activity, the board considers document E2 as representing the closest prior art.

11. Given that the prokaryotic expression of proteins having a TGF beta-like domain containing seven cysteine residues, i.e. BMP-2A and BMP-3, did not according to document E2 result in active proteins (point 10 above), the problem to be solved is seen in the provision of a process for the expression of osteogenic proteins having a TGF beta-like domain containing seven cysteine residues in prokaryotic host cells, which results in active proteins.

12. The solution to this problem as stated in claim 1 is a process which differs from the one disclosed in document E2 in that it includes the additional step of "refolding the expressed protein by oxidation" (part (d) of claim 1).

Plausibility that the problem is solved

12.1 According to the case law of the boards of appeal, the definition of an invention as being a contribution to the art presupposes that the solution to the technical problem is not merely put forward in a claim, but that either the disclosure in the application or the patent, respectively, in combination with or the common general knowledge alone made it plausible that the solution put forward in a claim is indeed a genuine solution to the problem. (T 1329/04 of 28 June 2005, point 12 of the reasons; T 604/04 of 16 March 2006, point 5 or the reasons; T 665/05 of 10 October 2006, point 14 of the reasons.)

13. At the oral proceedings the appellant argued that this was doubtful in the present case because there was only one example of the prokaryotic expression of an active osteogenic protein. Moreover, the description of how the expression was carried out was meagre, in particular with regard to the refolding conditions.

13.1 With regard to the latter argument based on the paucity of detail on how to carry out the production process, the board notes that, in line with the rationale of the decisions cited above, the question to be answered in this context is not whether or not the information in the patent is sufficient to enable the skilled person to carry out the claimed invention. The question, rather, is whether or not it can plausibly be stated, in view of the evidence from the disclosure in the patent and/or the common general knowledge, that the claimed subject-matter achieves the intended result.

13.2 Thus, it has to be decided whether or not the evidence available in the present case makes it plausible that the process according to claim 1 is indeed suited for the production of active osteogenic proteins.

13.3 Steps (a) to (c) of the process characterised in claim 1 are well known steps commonly used to express heterologous proteins in prokaryotic host cells. Step (d) is a well known measure for the recovery of active proteins after prokaryotic expression (for example, document E5). Moreover, it is undisputed that the disclosure in paragraphs [0080],[0081][0086 to 0088] and [0116] demonstrates that a process comprising the steps of the process according to claim 1 results in

the active osteogenic protein OP-1. On this basis and in the absence of evidence from the appellant, the board is satisfied that the problem as determined in point 11 above is solved by the subject-matter of claim 1.

The process claimed in independent claim 5 relates to the production of an osteogenic device and contains, by comparison with the process according to claim 1, the additional step of disposing the active protein in a matrix. In the absence of evidence to the contrary, the board is also satisfied that the process of claim 5 achieves the intended effect because matrices for disposing proteins are well known and also that the osteogenic activity of proteins embedded in such a matrix is retained (for example, document D10).

Obviousness

14. The appellant argued that it would be evident to the skilled person that the reason for the inactivity of the proteins BMP-2A and BMP-3 disclosed in document E2 was their aggregation in inclusion bodies, which are, as is well known, regularly formed after expression of foreign proteins in prokaryotic host cells (for example, document E5). The skilled person was aware of means, including oxidation, for recovering active proteins from inclusion bodies (documents D3 to D6). Consequently, it was an obvious measure to include the step of refolding by oxidation in the process disclosed in document E2 in order to solve the problem in question.

14.1 A question arising in view of the appellant's argument is whether or not the skilled person would have gathered from the prior art that the osteogenic proteins disclosed in document E2, BMP-2A and BMP-3, or proteins comprising a TGF-beta-like domain containing seven cysteines, are present in inclusion bodies after expression in prokaryotic cells.

14.2 In the only passage relating to prokaryotic expression of osteogenic proteins and the activity of the resulting products, document E2 reads as follows (page 1531, second column):

"Cartilage formation is also induced by recombinant BMP-1 expressed in E. coli. A 50-kD, NH_2-terminal fragment of BMP-1 was expressed in E. coli, solubilized from washed inclusion bodies and purified by heparin-Sepharose affinity chromatography to approximately 50 percent purity, although the amount of BMP-1 in an active conformation is not known. When 300 or 100 ng of this material was implanted in vivo, comparatively large areas of cartilage were formed (Fig. 5, A and B). Implantation of only 10 ng resulted in significantly less cartilage formation (Fig. 5C), while a similarly prepared negative control of the 30kD-COOH-terminal BMP-1 fragment had no activity (Fig. 5D). No activity was seen in similar experiments with various constructions of BMP-2A or BMP-3."

14.3 In the board's view, the skilled person would have understood that the expression "similar experiments" mentioned in the last sentence of the passage cited above refers to the activity tests reported in the penultimate sentence and not to the experiments carried

out for the purification of prokaryotic BMP-1. Therefore, the skilled person would not have inferred from the cited passage that BMP-2A and BMP-3, like BMP-1, are present in the form of inclusion bodies.

The disclosure in document E2 that BMP-1 is structurally unrelated to BMP-2A and BMP-3 and belongs to a different protein family is a further reason for ruling out such an interpretation (document E2, page 1533, first column).

14.4 Moreover, the formation of inclusion bodies after prokaryotic expression of BMP-2A and BMP-3 can be derived neither from document D1 (see points 2 to 2.6 above) nor from document E1, which does not even indicate whether recombinant expression was carried out in prokaryotic or eukaryotic cells. The latter document is therefore not relevant in this context.

14.5 Document "Nr.14" (referred to under "Sonstige Beweismittel" in the notice of opposition) relates to inhibin, which, like BMP-2A and BMP-3, is a member of the TGF-beta protein family (bottom of column 32, continued in column 33). However, in the worked examples only recombinant expression of inhibin in eukaryotic CHO cells is demonstrated. Therefore, this document too is not relevant to the present case.

14.6 The appellant argues (point 14 above) that the skilled person would have assumed the formation of inclusion bodies after prokaryotic expression of BMP-2A and BMP-3 on the basis of the common general knowledge that inclusion bodies are regularly formed in these circumstances.

14.7 However, the skilled person knows, for example from document E5, that inclusion bodies are not the only form in which proteins are found after prokaryotic expression, but that they may also be soluble or partially insoluble/soluble (point 2.4 above).

A similar teaching is also found in document D3 on page 3, lines 5 and 6:

"Under some conditions, and for some proteins, these heterologous proteins are precipitated in the cell as "refractile" bodies".

14.8 With regard to those proteins which contain cysteine residues, such as BMP-2A or BMP-3, the following is noted in document D4 on page 13842, second column:

"It is not unusual to find recombinant proteins, expressed in E. coli, in insoluble bodies of proteins known as refractile bodies (23, 25, 26). This has been found to occur most often with proteins that contain cysteine residues. Generally, when these proteins are extracted from cells, many of the disulfide bonds are formed incorrectly, causing the protein to be insoluble, inactive and often times aggregated."

14.9 However, this disclosure, which could be considered at least to imply a high probability of the formation of inclusion bodies in the case of cysteine-containing proteins, has to be balanced by the disclosure in Table 1 of document E5. The table indicates inter alia the number of cysteine residues and the solubility properties after expression in E. coli of thirty-three

eukaryotic proteins. It can be seen that the solubility differs even among proteins containing a similar number of cysteine residues. For example, urogastrone or calf prochymosine, which both have six cysteine residues, are present in the pellet after centrifugation, i.e. they are insoluble. Complement C5a and MMLV reverse transcriptase containing seven and eight cysteine residues respectively are partly soluble, while interferon alpha containing five cysteine residues is soluble. In contrast, the kappa-light chain of immunoglobulin G, another protein which also contains five cysteine residues, is insoluble.

14.10 As to predictions of the form in which proteins are found after expression in prokaryotic cells on the basis of structural characteristics, the following is reported in document E5, page 8, first column, last full paragraph:

"Such studies have led to the conclusion that the amino acid sequence of polypeptides contains the information required for folding (Anfinsen, 1973). Why is it, therefore, that eukaryotic polypeptides synthesized in E. coli and having the correct amino acid sequence fail to fold correctly? Insolubility does not result just because the proteins are expressed at a high percentage of total cell protein, as was observed for overexpressed E. coli proteins. There are examples of eukaryotic polypeptides expressed to levels of 1% or less which are insoluble (Table 1)."

The subsequent paragraph states:

"The mechanism by which proteins fold in vivo is still unknown. From studies in vitro it is evident that the amino acid sequence of each protein contains the information required for folding, but it is not apparent which residues specify the folding information. Another consideration is what influence, if any, the chemical environment within the cell has on protein folding. In the absence of such information it is only possible to speculate why some eukaryotic polypeptides fail to fold correctly in E.coli."

14.11 Thus, in the board's view, it follows from the observations in points 14.7 to 14.10 above that the skilled person would be aware that, generally, predictions about the form of a given protein after expression in prokaryotic host cells are not possible. The skilled person would not therefore conclude from the disclosures in the prior art that either BMP-2A or BMP-3 or, generally, proteins comprising a TGF-beta-like domain containing seven cysteines are present as inclusion bodies following prokaryotic expression.

14.12 Since the step of refolding in the context of recombinant prokaryotic expression presupposes the occurrence of inclusion bodies (points 2.4 and 2.5 above), the conclusion reached in point 14.11 has the consequence that the skilled person would also not have derived the step of "refolding the expressed protein by oxidation" as a means of obtaining active osteogenic proteins after prokaryotic expression in an obvious manner from the prior art.

14.13 Finally, the board has considered the possible argument that, in view of the common general knowledge in the

field of cloning, and in particular the cloning of eukaryotic genes in prokaryotic host cells, the skilled person was routinely aware of a number of reasons for the failure to obtain active proteins after expression, such as deficient cloning constructs, unsuitable host cells, inclusion bodies, etc., and that therefore in such a situation the skilled person merely had to find out, i.e. to try and see, which of these possibilities was the reason for failure in the specific case of osteogenic proteins having a TGF-like domain containing seven cysteines, with the consequence that by doing so the skilled person would have inevitably arrived at the claimed subject-matter. However, according to the case law of the boards of appeal, a "try-and-see" situation is considered to exist if the skilled person, in view of the prior art teachings, had clearly envisaged a way of proceeding in the light of the problem to be solved, for example if he or she had already envisaged a group of compounds as candidates for achieving an effect, the presence of which then only has to be verified by routine methods (for example, decision T 1599/06 of 13 September 2007, point 20.2 of the reasons). In the present case, the skilled person is not in such a position because, as follows from the observations above, the reason for the lack of activity was not clear and therefore the one way among the many possible ways of solving the problem was not foreshadowed.

14.14 Therefore, the board concludes that the subject-matter of claim 1 and that of dependent claims 2 to 4 involve an inventive step. For the same reasons, an inventive step is also acknowledged for the subject-matter of claim 5, which comprises the process of claim 1 as one

of its features (point 3.1 above), and its dependent claims 6 to 9.

The requirements of Article 56 EPC are fulfilled.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

P. Cremona M. Wieser