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Datasheet for the decision of 29 September 2015

Case Number: T 1424/11 - 3.3.02

Application Number: 99920202.1

Publication Number: 1113807

IPC: A61K35/34, A61K48/00, C12N5/10, C12N5/06, C12N5/08

Language of the proceedings: EN

Title of invention:
CELL MEDIATED GENE DELIVERY USING MUSCLE DERIVED CELLS FOR TREATING MUSCLE- AND BONE-RELATED INJURY OR DYSFUNCTION

Patent Proprietor:
UNIVERSITY OF PITTSBURGH

Opponent:
Beck, Josef

Headword:
Muscle-derived cells/UNIVERSITY OF PITTSBURGH

Relevant legal provisions:
RPBA Art. 15(3)
EPC Art. 123(2)

Keyword:
Amendments - added subject-matter (yes)

Decisions cited:

Catchword:

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Case Number: T 1424/11 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 29 September 2015

Appellant: (Patent Proprietor)	UNIVERSITY OF PITTSBURGH 200 Gardner Steel Conference Center Pittsburgh, PA 15260 (US)
Representative:	Cowley, Catherine Mary Venner Shipley LLP 200 Aldersgate London EC1A 4HD (GB)
Respondent: (Opponent)	Beck, Josef Max-Eder-Ring 36 85283 Wolnzach (DE)
Representative:	Dehmel, Albrecht Dehmel & Bettenhausen Patentanwälte Herzogspitalstrasse 11 80331 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 19 May 2011 revoking European patent No. 1113807 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	U. Oswald
Members:	T. Sommerfeld
	M. Blasi

Summary of Facts and Submissions

I. European patent 1113807, based on application 99920202.1, which was published as international application WO 1999/056785, was granted with 18 claims.

Independent claim 1 as granted read as follows:

"1. A method of isolating and purifying muscle-derived stem cells, comprising:
a) plating dissociated muscle cells in a first collagen-coated container;
b) passaging the cell supernatant to a new collagen-coated container after a portion of the cells have adhered to the first collagen-coated container;
c) repeating step (b) at least four times, and
d) isolating the adherent cells present after the at least fourth passage;
wherein said isolated cells have characteristics of muscle-derived stem cells comprising (i) higher levels of desmin staining relative to adherent cells from earlier passages, (ii) round phenotype, (iii) slower cell division than adherent cells from earlier passages; and (iv) pluripotency."

II. Opposition was filed against the granted patent, the opponent requesting revocation of the patent in its entirety on the grounds of lack of novelty and inventive step (Articles 54 and 56 EPC and Article 100(a) EPC), lack of sufficiency of disclosure (Article 100(b) EPC) and added subject-matter (Article 100(c) EPC).

III. During the proceedings before the opposition division, the patent proprietor requested that the opposition be rejected and the patent maintained as granted (**main**

request) or alternatively according to the first or second auxiliary requests (filed during oral proceedings before the opposition division).

Claim 1 of the **first auxiliary request** differed from claim 1 of the main request by the following amendments (insertions underlined, deletions struck through):

"1. A method ..., comprising:
a) ... first collagen-coated ~~container~~ flask;
b) ... new collagen-coated ~~container~~ flask after a ~~portion~~ 15 to 20% of the cells have adhered ...;
c) repeating step (b) serially ~~at least~~ four to six times, and
d) isolating the adherent cells present after the ~~at least~~ fourth to sixth passage;
wherein ..."

Claim 1 of the **second auxiliary request** differed from claim 1 of the main request by the following amendments:

"1. A method..., comprising:
a) ... first collagen-coated ~~container~~ flask;
b) ... new collagen-coated ~~container~~ flask after a ~~portion~~ 15 to 20% of the cells have adhered ...;
c) repeating step (b) serially ~~at least four times,~~; and
d) isolating the adherent cells present after the ~~at least fourth~~ fifth or sixth passage;
wherein ..."

IV. The opposition division revoked the patent under Article 101(2) and 101(3)(b) EPC.

It decided that all claim sets contravened the requirements of Article 123(2) EPC.

V. The patent proprietor (hereinafter appellant) lodged an appeal against that decision. With the statement of the grounds of appeal, the appellant requested that the decision be set aside and the case be remitted to the opposition division for further prosecution, and submitted three sets of claims as main, first and second auxiliary requests corresponding, respectively, to the main, first and second auxiliary requests considered in the appealed decision.

VI. With its letter of reply, the opponent (hereinafter, respondent) requested that the appeal be dismissed. As an auxiliary request, the respondent requested that the case be remitted to the opposition division for further prosecution.

VII. Summons for oral proceedings before the board were issued, scheduling oral proceedings for 29 September 2015.

VIII. With fax dated 28 September 2015, the appellant announced that it would not be attending oral proceedings.

IX. Oral proceedings before the board took place as scheduled, in the absence of the appellant. At the end of the oral proceedings, the chairman announced the decision of the board.

X. The appellant's submissions may be summarised as follows:

The terms "flask" and "container" were equivalent, as evidenced by exhibit 1, the dictionary's definition of "flask", and the person skilled in the art would consider the reference to a "collagen-coated container" in claim 1 of the main request to be directly and unambiguously derivable from the references to a "collagen-coated flask" in Examples 1 and 11 of the application as filed. The feature "passaging the cell supernatant (...) after a portion of the cells have adhered" found a basis in Example 1, on page 43 at lines 3 to 19, and in Example 11, on page 100 at lines 4 to 23. The skilled person, a cell biologist familiar with tissue culture techniques, would understand that both examples were intended to illustrate that the preplating technique used for purifying primary myoblasts required cell supernatant to be passaged to a new collagen-coated container after a portion of the cells had adhered; otherwise it would not be possible to separate fibroblasts from primary myoblasts on the basis of their differential adherence characteristics. Original claim 76 also disclosed such a method that involved serial passaging of cells without limitation to specific time periods, as could be derived from the fact that original claim 78, dependent on claim 76, required such time periods (meaning that claim 76 did not). As regards the feature "repeating step (b) at least four times", a basis was again to be found in Example 1, on page 43, and in Example 11, on page 100, which indicated that the "serial plating" technique was repeated six times overall, thus yielding six preplates in total, i.e. PP#1 to PP#6 (page 100, lines 18 to 19). Thus isolation of the adherent cells present after the at least fourth passage in step (d) of claim 1 could only be interpreted as isolation of adherent cells present in preplate 4 (PP#4). In combination with the disclosure

of Example 11, on page 100 at lines 19 to 27, and of Example 5, from page 67, line 30 to page 68, line 8, disclosing that the muscle-derived stem cells expressed high levels of desmin and that the first significant increase in the percentage of desmin-positive cells occurred between preplate #3 and preplate #5, i.e. in preplate #4, it would be clear to the skilled person that such desmin-expressing muscle-derived stem cells could be isolated from the at least fourth preplate; it would also be immediately evident for the skilled person that further enrichment in desmin-positive cells, above the level of 80% found in PP#6, should be possible and that the isolation of six preplates in Examples 1 and 11 was intended to be illustrative only. This was confirmed further by original claim 78, which was directed to a method that comprised isolating more than 6 preplates.

As regards the first auxiliary request, basis for the feature "repeating step (b) serially four to six times" and "isolating the adherent cells after the fourth to sixth passage" was also present in Example 1, page 43, and Example 11, page 100. Contrary to the conclusions of the opposition division that "the repetition of the passaging step six times would yield a population of cells corresponding to the eighth preplate", the above mentioned passages could only be interpreted as that the step of passaging the cell supernatant to a new collagen-coated flask after 15 to 20% of the cells had adhered to the first collagen-coated flask should be carried out four to six times in total, so as to obtain preplates four to six, i.e. PP#4 to PP#6.

As regards the second auxiliary request, the feature "isolating the adherent cells present after the fifth or sixth passage" was also disclosed in Examples 1 and

11 of the application as originally filed, which disclosed that six preplates in total were isolated by the serial preplating technique, i.e. PP#1 to PP#6, each preplate being associated with its own passaging step. Also Example 5, on page 68 at lines 3 to 15, specifically referred to preplates #5 and #6, the muscle-derived stem cells having been also purified by the preplating technique disclosed in Example 1 (page 62, lines 28 to 30).

XI. The respondent's arguments may be summarised as follows:

The definition cited by the appellant was evidence that "container" was indeed different from "flask". As regards the feature "passaging the cell supernatant (...) after a portion of the cells have adhered to the first collagen-coated container", Example 1 disclosed explicit conditions for preparation of muscle-derived stem cells (e.g. page 43, lines 3 to 10) which were not reflected in claim 1. Also page 43, lines 12 to 17, disclosed distinct numbers of preplates performed at defined passaging time intervals, which were not included in claim 1. Example 11, page 100 at lines 4 to 23, also disclosed distinct conditions for the preparation of the cell suspension and referred to 15-20% of cells that adhered to the flask, none of which were in claim 1. Neither of original claims 76 and 78 included a step of "passaging the cell supernatant (...) after a portion of the cells have adhered to the first collagen-coated container". The feature "repeating step (b) at least four times" in claim 1 was not disclosed in Example 1, which was restricted to a general teaching on the preplating technique as a method to enrich a muscle-derived cell population. Example 11, page 100 at lines 4 to 23,

described the preplating technique in more detail and stated that the procedure was repeated six times (page 100, lines 14 to 19): this was however no disclosure for four-, five-, seven- or eight-fold repetitions, all of which were encompassed by claim 1, step (c). Example 5 (page 67, line 30 to page 68, line 15) disclosed the amounts of cells positively stained for desmin in various preplates; it did not provide disclosure for step (c) of claim 1. Neither of original claims 76 and 78 disclosed a passaging step, let alone its four-fold repetition.

As regards the first and second auxiliary requests, when using the preplating technique according to Example 11 (in particular page 100, lines 4 to 19) and repeating the passaging step from PP#1 to PP#2 six times, six more passages would be performed and thus six more preplates would result, namely PP#3 to PP#8. However preplates higher than PP#6 were not disclosed in Example 11 or elsewhere in the original application. Also Example 5 disclosed only a maximum of six preplates (e.g. page 68, lines 8 and 12, and page 69, line 8).

XII. The appellant requested in writing that the decision under appeal be set aside and that the case be remitted to the opposition division for further prosecution on the basis of the set of claims of the main request or, alternatively, of the first or second auxiliary requests, all filed with the statement of the grounds of appeal.

The respondent requested that the appeal be dismissed or, alternatively, that the case be remitted to the opposition division for further prosecution.

Reasons for the Decision

1. The appeal is admissible.

2. The oral proceedings before the board took place in the absence of the appellant who had been duly summoned but decided not to attend.

Under Article 15(3) RPBA the board is not obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying only on its written case. Thus, for reasons of procedural economy, the board decided to continue the proceedings in the absence of the appellant in accordance with Rule 115(2) EPC. The principle of the right to be heard pursuant to Article 113(1) EPC is observed since that Article only affords the opportunity to be heard and, by absenting itself from the oral proceedings, a party gives up that opportunity.

3. Main request - Article 123(2) EPC

3.1 Article 123(2) EPC stipulates that the European patent application or the European patent may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed.

3.2 In accordance with established case law of the boards of appeal, the relevant question to be decided in assessing Article 123(2) EPC is whether the skilled person would derive the subject-matter as amended directly and unambiguously from the application as

filed, meaning that the amendments must not result in the introduction of technical information which a skilled person would not have objectively derived from the application as filed.

Moreover, the content of a document as originally filed may not be seen as a reservoir of features from which features pertaining to separate embodiments can be combined in order to artificially create a particular embodiment.

3.3 As stated by the opposition division in the appealed decision, the granted claims are derived from original claims 76 to 80.

Original claim 76 reads:

"76. A method of isolating and purifying muscle-derived stem cells, comprising:
a) plating dissociated muscle cells on a collagen-coated substrate;
b) isolating muscle-derived cell populations which adhere to said substrate at successive time intervals following said plating step a); and
c) determining the characteristics of the isolated cell populations to identify muscle-derived stem cells."

3.4 Claim 1 of the main request (see point I above for its wording) thus differs from this claim in that: a "collagen-coated container" rather than a "collagen-coated substrate" is used in step (a); steps (b) and (c) have no counterpart in the original claim; the isolation step is defined differently, namely it is "isolating the adherent cells present after the at least fourth passage" (step (d)); and present claim 1 specifically defines the characteristics of muscle-

derived stem cells in its epilogue. According to the appellant, a basis for these amendments is to be found in Examples 1, 5 and 11.

3.5 The board notes that claim 1 of the main request and claim 76 as originally filed actually relate to two different methods to isolate and purify muscle-derived stem cells. While the first involves repeated passaging of the cell supernatant (at least four times) after a portion of the cells have adhered and then isolating the adherent cells, the second one involves isolating the adherent cells at successive time intervals, without any restrictions as to the presence and number of adherent cells. The method of original claim 76 is exemplified in Example 1 (page 43, lines 10 to 17), which also defines the different preplates as representing cell populations that adhere at different time intervals and does not even refer to any passaging of cell supernatant. Thus the board considers that neither originally filed claim 76 nor Example 1 can provide a basis for present claim 1. Likewise no basis can be derived from Example 5, which also only refers back to the method of Example 1.

3.6 Notwithstanding the above conclusions, the board notes that, even if original claim 76 were taken as a basis for present claim 1, the features added to claim 76 would still not be disclosed in the application as filed, let alone in combination with the other features of the claim.

3.7 There is no disclosure of a "collagen-coated container" in the application as filed. Instead, originally filed claim 76 refers to "collagen-coated substrate" while Examples 1 and 11 refer to a "collagen-coated flask". The board notes that, while a flask is certainly a

container, it is nevertheless a container with specific characteristics, as is made clear by the definition of "flask" given in Exhibit 1 (entry for "flask" in The Merriam Webster Online Dictionary) and cited by the appellant: "a container often somewhat narrowed toward the outlet and often fitted with a closure". Hence the concept of container encompasses flask but is broader in meaning. The term "collagen-coated container" in claim 1 is thus a generalisation of the originally disclosed term "flask", and such a generalisation finds no basis in the application as originally filed.

3.8 There is also no disclosure in the application as filed of claim 1's step "b) passaging the cell supernatant to a new collagen-coated container after a portion of the cells have adhered to the first collagen-coated container". Example 1 on page 43, lines 10 to 19, makes no reference at all to supernatant; instead it merely discloses that "[d]ifferent cell populations of muscle-derived cells were isolated based on the number of preplates performed on collagen-coated flasks" (lines 11 and 12), and goes on to explain how each different preplate is formed: "Preplate #1 (PP#1) represented a population of muscle-derived cells that adhered in the first hour following isolation; PP#2 represented a population of muscle-derived cells that adhered in the next two hours; PP#3 represented a population of muscle-derived cells that adhered in the next 18 hours; and the subsequent preplates were obtained at 24 hour intervals (PP#4-6)" (lines 12 to 17). Thus very specific time periods are given for each preplate, rather than just a reference to "after a portion [undefined] of the cells have adhered to the first collagen-coated container", as now in the claim. Example 11 does indeed refer to serial passage of supernatant, but further states that said passage is

done "after 15-20% of the cells adhered", which is not the same as "after a portion of the cells adhered". Thus Example 11 cannot constitute a basis for this amendment either. The appellant has also referred to original claim 78 as providing evidence that the restriction to the specific time periods mentioned in Example 1 was not part of original claim 76. The board agrees that said restriction is indeed not present in original claim 76, but notes that claim 76 provides no basis whatsoever for the amendment at issue: rather, the corresponding step in claim 76 reads "isolating muscle-derived cell populations which adhere to said substrate at successive time intervals following said plating step a)", which is actually a general disclosure of the method of Example 1. There is thus no direct and unambiguous disclosure in the application as filed for step (b) of claim 1 of the main request.

3.9 Finally, there is also no disclosure in the application as filed for step (c) of claim 1: "repeating step (b) at least four times". Contrary to the appellant's arguments, a disclosure of a total of six preplates in Example 1 (page 43) cannot constitute a basis for this amendment, which corresponds to a range of four or more passages (with no upper limit). The same is also true for Example 11, which again refers to six preplates and not more. Whether it could be evident for the skilled person that more myoblast-enriched populations could be obtained after more than 6 passages is a question of obviousness and not of direct and unambiguous disclosure: this argument is thus of no relevance in the assessment of Article 123(2) EPC. The fact that original claim 78 discloses 8 time intervals for step (b) of claim 76 still does not provide disclosure for "repeating step (b) at least four times": first, step (b) of claim 1 of the main request is not

identical to step (b) of claim 76 as originally filed (see above), and, second, this feature can only provide a basis for the eight specifically defined time intervals and for nothing else.

3.10 Claim 1 of the main request thus does not comply with Article 123(2) EPC.

4. First auxiliary request - Article 123(2) EPC

4.1 In claim 1 of this request, the term "container" has been replaced by "flask"; step (b) has been amended to read "after a 15 to 20% of the cells" instead of "after a portion of the cells"; step (c) has been amended to "repeating step (b) serially four to six times"; and step (d) refers now to isolation of the adherent cells present "after the fourth to sixth passage".

4.2 The feature of passaging the cell supernatant after 15 to 20% of the cells have adhered is disclosed in Example 11, as discussed above in relation to the main request. This example however discloses that the procedure (i.e. the preplating) "was repeated six times, yielding six preplates" (page 100, lines 18 and 19). There is no disclosure of repeating four to six times (step (c)), let alone of isolating the adherent cells after the fourth to sixth passage (step (d)). Thus the method of Example 1 involves passaging six times and there is no individualised disclosure of a method involving only four or five passages, as is now also encompassed by the claim. Example 1 cannot provide a basis for this claim either, because, as mentioned above, it actually relates to a different method wherein the preplating is performed not after a given number of adherent cells is present but rather after defined time intervals.

4.3 Claim 1 of the first auxiliary thus also fails to comply with Article 123(2) EPC.

5. Second auxiliary request - Article 123(2) EPC

5.1 Claim 1 of this request differs from claim 1 of the first auxiliary request in that step (c) reads "repeating step (b) serially" and step (d) is directed to isolation of the adherent cells after the fifth or sixth passage.

5.2 For the same reasons as discussed above in relation to the first auxiliary request, also this claim finds no basis in the application as filed. Example 11 is the only disclosure of passaging after 15 to 20% of the cells have adhered, but it provides no basis for isolation after the fifth or sixth passage. Also Example 5 (page 68, lines 5 to 8) does not disclose isolation after the fifth or sixth passage; not only is there no disclosure in this Example for passaging after adherence of 15 to 20% of the cells, but also there is no disclosure of isolation after the sixth passage.

5.3 Claim 1 of the second auxiliary request thus also fails to comply with Article 123(2) EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

N. Maslin U. Oswald

Decision electronically authenticated