The opinion in support of the decision being entered today was not written
for publication and is not binding precedent of the Board.

Paper No. 44

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte WALLACE H. COULTER, ROBERT K. ZWERNER
ROBERT J. SCHMITTLING and THOMAS R. RUSSELL

Appeal No. 2002-2279
Application No. 08/556,667[1]

ON BRIEF

Before SCHEINER, GRIMES and GREEN, Administrative Patent Judges.

SCHEINER, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the final rejection of claims 4-20 and 28; claims 21-27 and 29 are also pending, but have been withdrawn from consideration; claims 1-3 have been canceled. Claims 4 and 5 are representative:

4. A method of enhancing a selected population or subpopulation of cells of interest in a fluid sample having a plurality of populations of cells, comprising:
providing a plurality of particles having a density at least two times the density of the cells of interest and sufficient to provide differential gravity settling, said particles having bound thereto a reactant which specifically binds to the cells or biological particles of at least one preselected population or subpopulation not of interest;
mixing a portion of said sample with said particles to bind said particles to said cells or biological particles of said preselected population or subpopulation without substantially physically damaging said cells of said selected population or subpopulation of interest;
differential gravity settling said particles with said bound population or subpopulation of cells or biological particles in said sample portion, wherein settling is

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[1] Application for patent filed November 13, 1995. According to appellants, this application is a continuation-in-part of application serial no. 08/228,791, filed April 15, 1994, now U.S. Patent 5,576,185.

accomplished by gravity separation alone;

removing at least a portion of the resultant supernatant of said sample portion including said selected population or subpopulation of said cells of interest from said particles and said bound population or subpopulation of cells or biological particles;

providing a second plurality of particles having a density of at least two times the density of the cells of interest and sufficient to provide differential gravity settling, said particles having bound thereto a reactant which specifically binds to the cells or biological particles of at least one preselected population or subpopulation not of interest;

mixing at least a portion of said removed supernatant of said sample portion including said selected population or subpopulation of cells of interest with said second plurality of particles to bind said second plurality of particles to said second preselected population or subpopulation of cells or biological particles without substantially physically damaging said selected population or subpopulation of cells of interest;

differential gravity settling said second set of particles with said bound population or subpopulation of cells or biological particles in said supernatant sample portion, wherein settling is accomplished by gravity separation alone; and

removing at least a portion of the resultant second supernatant including said selected population or subpopulation of cells of interest from said second set of particles and said bound population or subpopulation of cells or biological particles and recovering at least fifty percent of said selected population or subpopulation of cells originally in said supernatant sample portion.

5. A method of enhancing a selected population or subpopulation of cells of interest in a fluid sample, comprising:

(a) providing a plurality of particles having a density at least two times the density of the cells of interest and sufficient to provide differential gravity settling, said particles having bound thereto at least one reactant which specifically binds to at least one preselected population or subpopulation of cells or biological particles not of interest;

(b) mixing said sample with said particles to bind said particles to said preselected population or subpopulation without substantially physically damaging said population or subpopulation to be enhanced;

(c) permitting said particles and said particles bound to said preselected population or subpopulation of cells to settle via differential gravity settling, leaving a supernatant, wherein settling is accomplished by gravity separation alone; and

(d) separating at least a portion of the supernatant comprising said population or subpopulation to be enhanced from said settled particles and said settled particles bound to said preselected population or subpopulation not of interest.

The references relied on by the examiner are:

Giaever	4,115,535	Sep. 19, 1978
Grenier et al. (Grenier)	4,788,136	Nov. 29, 1988
Pry et al. (Pry)	5,229,268	Jul. 20, 1993

Falkenburg et al. (Falkenburg), “Selective Removal of Clonogenic Neoplastic B Cells from Human Bone Marrow Using Anti-HLA-DQ Antibodies and Complement,” Exp. Hematol., Vol. 14, pp. 101-107 (1986)

Reynolds et al. (Reynolds), "Model System for Removing Neuroblastoma Cells from Bone Marrow Using Monoclonal Antibodies and Magnetic Immunobeads," Cancer Research, Vol. 46, pp. 5882-5886 (November 1986)

Kessler et al. (Kessler), "Large-Scale Purification and Characterization of CD34-Positive Hematopoietic Progenitor Cells," Blood, Vol. 70 (Suppl. 1), Abstract No. 321a (1987)

Patel et al. (Patel), "Use of Density Perturbation to Isolate Immunologically Distinct Populations of Cells," Journal of Immunological Methods, Vol. 163, pp. 241-251 (1993)

The claims stand rejected as follows:

I. Claims 4, 5, 8, 9, 11-14, 18, 19 and 28 under 35 U.S.C. § 103 as unpatentable over Grenier and Reynolds.

II. Claims 4, 5, 8, 9, 11-14, 18, 19 and 28 under 35 U.S.C. § 103 as unpatentable over Pry and Reynolds.

III. Claims 6, 7 and 9 under 35 U.S.C. § 103 as unpatentable over Grenier, Pry, Reynolds and Kessler.

IV. Claim 15 under 35 U.S.C. § 103 as unpatentable over Grenier, Pry, Reynolds and Giaever.

V. Claims 16 and 17 under 35 U.S.C. § 103 as unpatentable over Grenier, Pry, Reynolds and Patel.

VI. Claims 10 and 20 under 35 U.S.C. § 103 as unpatentable over Grenier, Pry, Reynolds, Kessler and Falkenburg.

BACKGROUND

"The enhancement of a population or a subpopulation of a sample such as hematopoietic stem cells (HSC) can be utilized for many types of applications . . . In addition to enhancement . . . , it is often necessary to remove undesired populations or subpopulations. For example, . . . [i]f the bone marrow is to be purged of [non-Hodgkin's B lymphoma cells] and reinfused into the patient, it is important that the bone marrow be completely purged and that the bone marrow be not otherwise damaged" (specification, pages 1 and 2).

> Currently, one approach is to utilize a plurality of magnetic microspheres, typically formed of a polymer based magnetic material of a relatively low density . . . [T]he microspheres are mixed with the bone marrow or blood and . . . are intended to be maintained in suspension in the sample and consequently are designed for very slow or substantial elimination of gravity settling in the sample suspension.
>
> The magnetic microspheres have at least one antibody bound thereto specific to the population or subpopulation desired to be removed . . . For purging blood or bone marrow, a sample would be mixed with a plurality of the antibody bound microspheres and then placed in a magnetic field. The remaining sample or supernatant is removed while the microspheres are held in the magnetic field. This procedure typically must be repeated, since a single purging step generally will not deplete a sufficient percentage of the undesired population . . .
>
> The magnetic removal procedure . . . removes a number of cells non-specifically from other populations during each removal step . . . A single removal step results in a varying yield of a relatively low percent with each succeeding step also reducing the yield.

Specification, pages 2-3.

According to appellants, the present invention solves this problem by mixing the sample with particles "substantially more dense than the cells, at least on the order of two (2) to three (3) times more dense than the cells" and allowing "the particles and the targeted populations bound thereto [to] settle differentially through the sample [ ], leaving the unbound/non-targeted populations in suspension" (specification, page 10). "An advantage of the dense particles [ ] is that they differentially will gravity settle through the sample [ ] following mixing without substantial trapping of non-selected or non-targeted cells" (id., pages 9-10), "provid[ing] a high yield of the cells of interest even after multiple removal steps" (id., page 6).

DISCUSSION

The examiner rejected all of the claims as obvious over the prior art, and each of six the rejections relies, at least in part, on the examiner's proposed combination of Reynolds with Grenier or Pry, so we will discuss all six rejections together.

Reynolds describes removing neuroblastoma cells from bone marrow by incubating neuroblastoma cells with neuroblastoma cell-specific monoclonal antibodies, mixing the antibody-coated cells with a bone marrow sample, capturing the antibody-coated neuroblastoma cells with goat anti-mouse immunoglobulin-coated magnetic microbeads, removing microbead-captured neuroblastoma cells from the bone marrow sample by passing the sample over a magnet, and recovering the remaining bone marrow cells from the purged sample. "The efficacy of tumor cell removal depended on the amount of monoclonal antibody bound to tumor cells and the immunobead/tumor cell ratio. In addition, two cycles of purging with both monoclonal antibodies and immunobeads was superior to one cycle. Using a cocktail of [ ] five antibodies, 3 to 4 logs of tumor cells could be depleted from marrow with good recovery of viable hematopoietic cells." Pages 5882 and 5883. While Reynold's magnetic immunobeads are described as "[p]olystyrene porous magnetic beads . . . 3 μm in diameter," no mention is made of the density of the beads.

Grenier describes a diagnostic immunoassay wherein excess labeled anti-analyte antibody is removed "by adding a solid phase material having immobilized thereon a compound having a preferential binding affinity for the labeled anti-[analyte] antibody" (column 3, lines 60-64). "The solid phase material can be fabricated from any number of synthetic materials . . . such as agarose, polystyrene, polyacrylamide, their derivatives or mixtures thereof" (column 4, lines 18-22) and "is present as a particle or matrix having a density greater than water" (column 4, lines 3-4), "such that a rapid dispersal by agitation and sediment by gravity is facilitated" (column 3, line 65 to column 4, line 2). Pry's disclosure is essentially the same as Grenier's in this respect, and is cited by the examiner for essentially the same reasons (Answer, pages 6 and 7).

If we understand the examiner's position, it is that it would have been obvious for one skilled in the art to enrich a selected population of cells in a sample by capturing unwanted cells on particles coated with binding partners specific for the unwanted cells (as taught by Reynolds), allowing the particles to settle by gravity separation alone (as taught by Grenier, and also by Pry), and recovering cells of interest from the supernatant, because Grenier teaches that gravity separation "is useful where traditional purification or removal means are not appropriate, such as [ ] centrifugation or filtration" (Answer, page 4), and Reynolds teaches that the use of particles in a negative cell selection process "provides a high level of recovery" and "does not substantially physically damage the [cells of interest]" (Answer, pages 5-6).

With respect to the requirement for "particles having a density of at least two times the density of the cells of interest" (claims 4 and 5, e.g.), the examiner appears to concede that none of the references teaches particles at least twice as dense as Reynold's bone marrow cells, but argues that the "claims simply recite that the particles are at least two times [as] dense[as] a cell[ ] where no particular type of cell is recited . . . [t]hus the particle can be twice as dense[ ] [as] some type of cells" (Answer, page 13). In any case, the examiner believes that "[t]he use of a known member of a class of materials, such as the particular size of a particle, in a process is not patentable if other members of the class were known to be useful for that purpose . . . [and n]o more than routine skill is involved in adjusting the density or size of component . . . to suit a particular starting material in order to achieve the results taught in the prior art" (id., pages 4-5).

Nevertheless, we agree with appellants that none of the references relied on by the examiner describes or suggests particles "having a density at least two times the

density of the cells of interest" (see e.g., claims 4 and 5) and we too conclude that the examiner has not made out a prima facie case of obviousness.

As explained in In re Kotzab, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316 (Fed. Cir. 2000):

> A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. [ ] Close adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one "to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher." [ ]

We have no doubt that the prior art could be modified in a manner consistent with appellants' specification and claims. The fact that the prior art could be so modified, however, would not have made the modification obvious unless the prior art suggested the desirability of the modification. In re Gordon, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984).

In this case, the claims on appeal place a specific limitation on the particles used in the claimed methods - the particles must have a density at least twice that of the cells of interest. The fact that the claims do not specify a particular "starting material" or cell of interest as a reference point for the density of the particles does not mean that the limitation can be met by any particles which happen to have a density twice that of some cell type. Assuming that one skilled in the art would have been motivated to use the methods and materials of Grenier or Pry to purge Reynolds' bone marrow cells of neuroblastoma cells, there must still have been some reason or suggestion in the prior art to use particles at least twice as dense as Reynolds' bone marrow cells (the "starting material" or reference point in this case) in order to meet the limitations of the claims.

We do not find evidence of such a reason or suggestion in the references relied

on by the examiner. Grenier's particles (and Pry's) need only have a "density and overall size . . . such that a rapid dispersal by agitation and sediment by gravity is facilitated" (Grenier, column 3 line 68 to column 4, line 2). The particles are described in an open-ended way as "having a density greater than water" (id., column 4, line 4), but there is nothing to indicate that any of the particles have anything approaching twice the density of Reynolds' bone marrow cells. Indeed, there would appear to be no dispute that the densest particle described by Grenier (and Pry) is composed of Trisacryl®, which, according to appellants, has "a density of about 1.12 ± 0.03 gm/cc" (Brief, page 5), while blood cells, i.e., bone marrow cells, "have a density of about 1.05 gm/cc" (id.). Since density is not a factor in Reynolds' method at all, and Grenier indicates that Trisacryl® particles are "easily suspended in solution yet sufficiently dense to rapidly sediment by gravity" (column 8, lines 24-25), we see no reason or suggestion, apart from appellants' specification, to modify the density of the particles to the extent required by the claims.

In our opinion, the only reason or suggestion to modify Reynolds, Grenier and Pry in the manner proposed by the examiner comes from appellants' specification. Accordingly, we find that the examiner has not established a prima facie case of obviousness for independent claims 4 and 5, the broadest claims on appeal. Giaever, Falkenburg, Kessler and Patel were cited with respect to limitations of some of the dependent claims, but do nothing to remedy the underlying deficiency of the examiner's proposed combination of Reynolds with Grenier or Pry, so we will not discuss them further.

The rejections of the claims under 35 U.S.C. § 103 are reversed.

REVERSED

	)
Toni R. Scheiner	)
Administrative Patent Judge	)
	)
	)
	) BOARD OF PATENT
	)
Eric Grimes	) APPEALS AND
Administrative Patent Judge	)
	) INTERFERENCES
	)
	)
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Lora M. Green	)
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