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Test report – Expert report

Determination of the resorptive potential of the wound dressings "curea P1" and "curea P2" with respect to whole blood, plasma, serum and physiological saline

1. Introduction and problem

Within the scope of this expert report, the resorptive potential of the wound dressings "curea P1" and "curea P2" (Curea Medical GmbH, Berlingerode) are to be tested with respect to the following wound-relevant liquids:

- o whole blood
- o plasma
- o serum
- o saline w = 0.9% (physiological)

In terms of the wound exudate, a high salt and protein composition is ideally to be expected. A wound exudate therefore corresponds most closely with the plasma fraction.
In fresh wounds, after-bleeding can occur, which, in light of wound clearance, can be desirable in some cases. Thus, examination of the resorptive potential of the wound dressings is particularly relevant in the case of whole blood, due to the fact that without sufficient resorption the liquid proportion of the blood (plasma) is resorbed, but it is possible for the blood cells in the wound to be enriched. Caused by haemolysis phenomena, enrichment of cell components of the blood can release different tissue kinins which stimulate inflammation. In addition, coagulation is initiated, which can delay the granulation phase of the wound.

In order to avoid these issues, it is pertinent that the wound dressings described above carry out a 1:1 resorption of blood.
The main focus in this expert report is therefore placed on blood resorption. In parallel, the resorption capacity of the wound dressings is compared with plasma, serum and physiological saline.

2. Method of determination

200ml of each of the liquids to be tested (whole blood, plasma, serum and physiological saline) is poured into crystallisation dishes with a diameter of 300mm.

Whole blood:
Whole blood material is used after inhibition of coagulation by sodium citrate. Citrate forms a dative bond with calcium ions (as chelate complex). Since calcium ions represent the coagulation factor IV and this is involved in all processes which stimulate coagulation, an anticoagulatory effect is generated by a complex bond of the calcium ions.

Plasma:
Whole blood material is mixed with EDTA potassium salt. EDTA forms a dative bond with calcium ions and magnesium ions and, therefore, in a similar way to citrate, initially generates an anticoagulatory effect. Because magnesium ions and calcium also ions are bound, enzymatic cofactors of the coagulation cascade are also deactivated, thereby protecting the coagulation proteins against changes (secondary coagulation phenomenon) and remain in the liquid phase.
The cell fraction is separated by centrifugation, the liquid phase of the blood with all proteins including the (inhibited) coagulation proteins remains in the supernatant.

Serum:
The whole blood material is natively mixed with glass beads. The foreign surface (glass) induces the extrinsic coagulation cascade, thereby binding the cell components together with the coagulation proteins as a red thrombus.
A salt solution which still contains non-coagulation proteins (soluble enzymes, albumin and globulins) remains in the supernatant.

Physiological Saline:
This solution represents the aqueous fraction of the blood only. The wound dressings to be examined (curea P1, curea P2) are first weighed and then placed into the liquid to be tested along with the resorption side.
After an interval of 60 minutes, the wound dressing is removed from the liquid and freed of adhesions by dabbing off. Finally, the mass after resorption is determined and the resorbed mass is determined by subtraction.

Each test liquid and each product is examined in one test with two test specimens in parallel.
As regards of the test liquid whole blood, the number of erythrocytes is determined using a microscope (after appropriate preparation of the blood using Hayem's solution consisting of isotonic sodium sulphate/mercury(II) chloride solution) before and after resorption (in the volume remaining in the crystallisation dish) using a Neubauer counting chamber. Thus, it can be determined if an enrichment of the erythrocytes fraction occurs in the remaining residual volume, caused by resorption. After resorption has taken place, the resorption core of the wound dressings treated with whole blood is opened after a time interval of 24 hours at 36℃. The resorption material is then assessed usin g a microscope. An aliquot is diluted 1:1 with Hayem's solution, subsequently one drop is spread across a microscope slide and a Pappenheim stain is carried out.
Thus, it can be determined whether, after resorption has taken place, haemolysis occurs in the resorption core within a time interval of 24 hours.

3. Results

3.1 Results of whole blood resorption:

Product:	Initial mass	Mass after resorption	Resorbed mass	N(Ery) before resorption	N(Ery) after resorption
P1(A)	4.52 g	72.83 g	68.31 g	$10.12 * 10^6$/µL	$8.21 * 10^6$/µL
P1(B)	4.44 g	71.20 g	66.76 g	$10.12 * 10^6$/µL	$8.77 * 10^6$/µL
P2(A)	4.73 g	68.75 g	64.02 g	$10.12 * 10^6$/µL	$9.22 * 10^6$/µL
P2(B)	4.68 g	70.75 g	66.07 g	$10.12 * 10^6$/µL	$9.28 * 10^6$/µL

Note: The number of erythrocytes (N(Ery)) in the test fluid was determined before and after the resorption.

Logarithm of the number of erythrocytes before resorption:

7.01

Logarithm of the number of erythrocytes after resorption:

		log.difference:
P1(A)	6.91	-0.10
P1(B)	6.94	-0.07
P2(A)	6.96	-0.05
P2(B)	6.97	-0.04

Average of the change of number of erythrocytes:

P1	6.93	-0.08
P2	6.97	-0.04

In none of the samples haemolysis was observed after exposition over 24 hrs. at 36℃.

3.2 Results of plasma resorption:

Product:	Initial mass	Mass after resorption	Resorbed mass
P1(A)	4.51 g	76.44 g	71.93 g
P1(B)	4.45 g	75.10 g	70.65 g
P2(A)	4.71 g	74.77 g	70.06 g
P2(B)	4.69 g	76.24 g	71.55 g

3.3 Results of serum resorption:

Product:	Initial mass	Mass after resorption	Resorbed mass
P1(A)	4.53 g	81.02 g	76.49 g
P1(B)	4.51 g	80.10 g	75.59 g
P2(A)	4.73 g	82.15 g	77.42 g
P2(B)	4.74 g	81.69 g	76.95 g

3.4 Results of saline resorption:

Product:	Initial mass	Mass after resorption	Resorbed mass
P1(A)	4.51 g	89.04 g	84.53 g
P1(B)	4.49 g	90.10 g	85.61 g
P2(A)	4.74 g	91.25 g	85.61 g
P2(B)	4.70 g	90.90 g	86.20 g

4. Interpretation and discussion

The results in point 3.1 show that in products “curea P1” and “curea P2”, a resorption of whole blood occurs at a ratio 1:1. An enrichment of cellular components of the blood in the volume remaining after resorption therefore does not take place. In contrast, a small decrease in the number density was observed (log 0.08 in case of curea P1 and log 0.04 in case of curea P2).
Over an exposition time of 24 hours at 36℃, haemol ysis of the resorbed whole blood does not take place, meaning that a liberalisation of tissue kinins, which could possibly diffuse back from the wound dressing into the wound, does not occur.
As expected, a higher total resorption capacity (points 3.2, 3.3 and 3.4) was determined in the cell-free liquids (plasma, serum and saline). The resorption capacity increases with decreasing protein content of the liquids due to the fact that, with decreasing protein content, the proportion of free water not bound in hydrate covers increases.

ATTENTION:
This translation has been prepared by the below signing employee of curea medical GmbH and is valid with the German text only. No content has been omitted or added or has been changed according to my best knowledge.

Berlingerode, 02. February 2012

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