A new animal-product-free, defined, and universal cell culture medium:

Easy to use, do-it-yourself and beneficial for 2D and 3D culturing of normal and cancer cells

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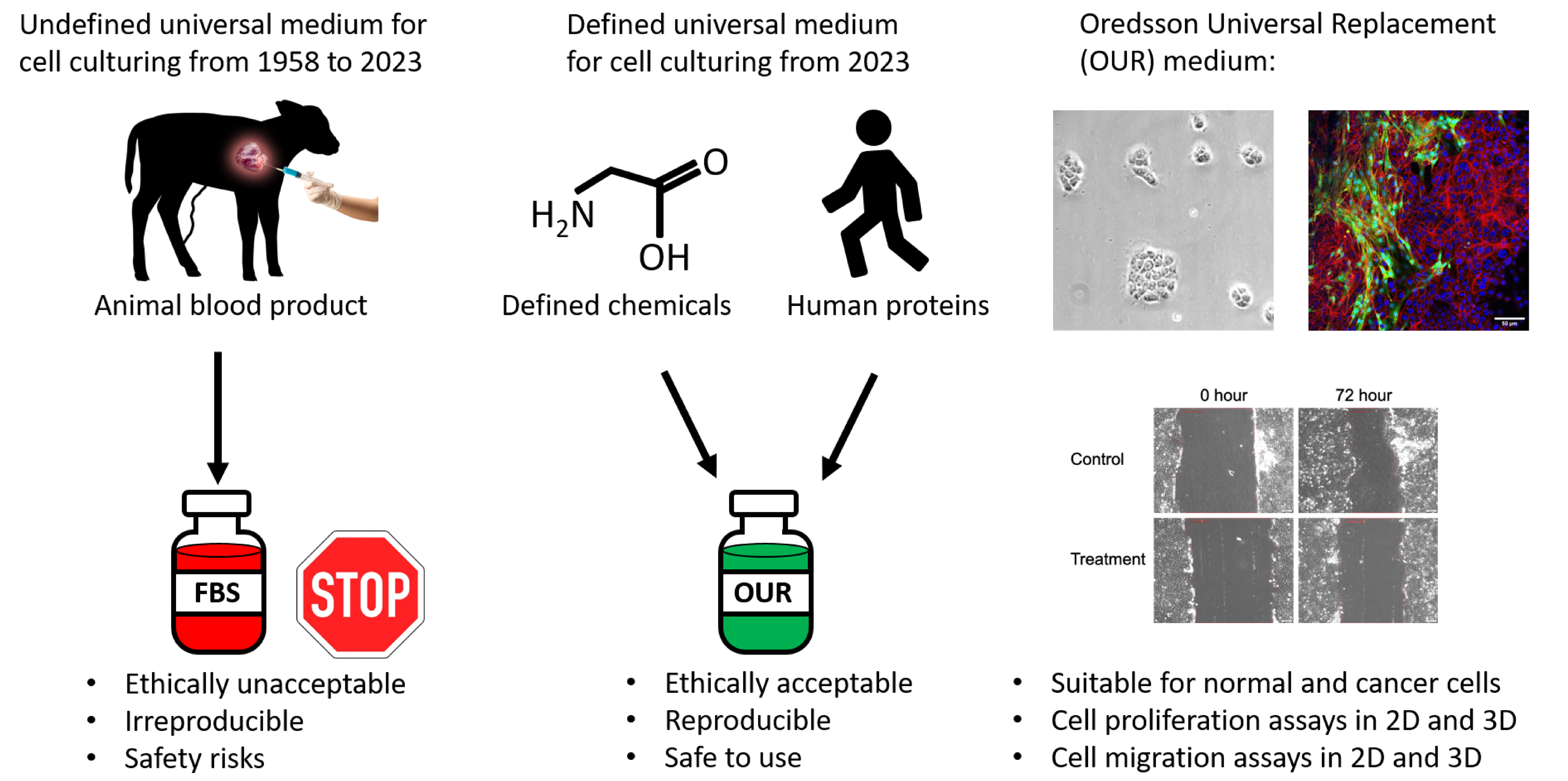
Introduction

Cell culturing methods are increasingly used to reduce and replace the use of live animals in biomedical research and chemical toxicity testing. Although cell culture methods support sparing live animals from being used in experiments, they often contain animal-derived components like fetal bovine serum (FBS).

FBS is added to cell culture media among other supplements to support cell attachment, spreading and proliferation. Safety, reproducibility and ethical problems with animal sera are widely acknowledged [1] and therefore worldwide efforts are ongoing to manufacture animal-product-free media. Now we provide the formulation of a new defined medium [2,3] with human-derived or recombinant proteins for long term routine culturing of regular and cancer cells as well as cryopreservation: **Oredsson Universal Replacement (OUR) Medium.**

OUR medium

- We provide growth and dose response curves of cells grown in two and three dimensions as well as applications such as cell migration [2]
- Cell morphology was studied in real time by phase contrast and phase holographic microscopy time-lapse imaging [2]
- The composition of the medium is listed on **Table 1**



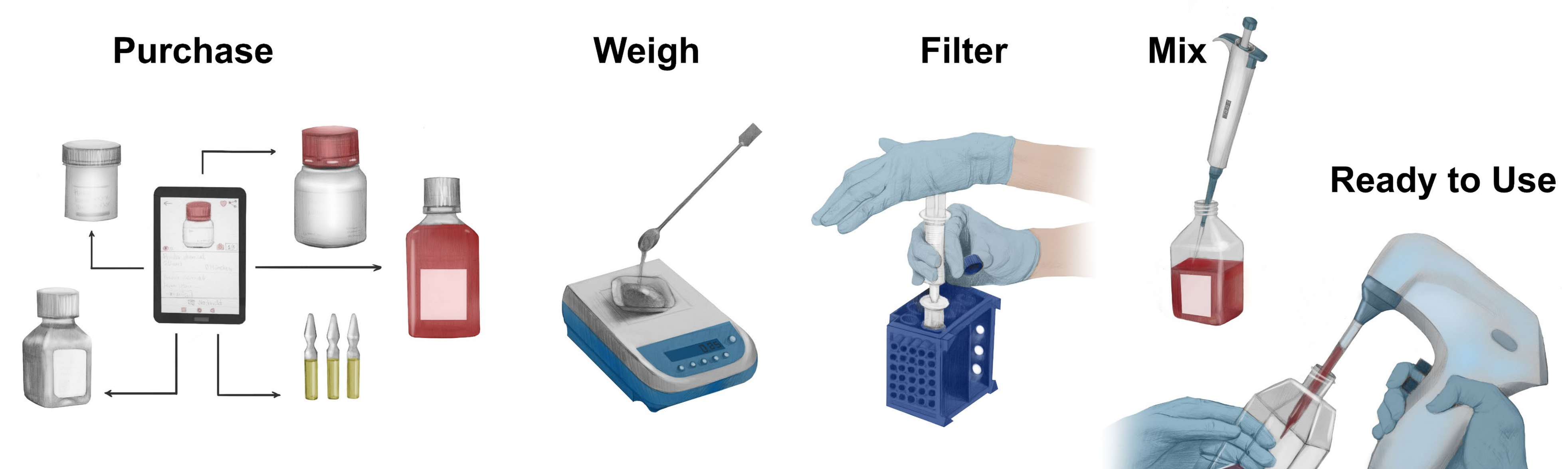
Why FBS must be replaced

The undefined nature of FBS combined with high batch-to-batch variability and risk of contaminations place it as an additional uncertainty factor introduced in cell culture.

Furthermore, its production involves blood retrieval from bovine fetuses, which either die shortly before or during the procedure without legal obligations to apply anesthesia or a certified killing method in compliance with animal welfare regulations.

Tested cell lines

OUR medium is successfully used with human normal and cancer-associated fibroblasts, human keratinocytes, several human breast cancer and pancreatic cancer cell lines, human colon cancer CaCo-2 cells and murine L920 fibroblasts as well as several other normal and cancer cells [2,3]. Many more are currently tested.



Schematic representation of steps to prepare the animal free defined cell culture medium

Table 1
Composition of the defined medium.

Medium component	Concentration in medium
Basal medium: DMEM / Hamś F12	-
Optional extra buffer: HEPES	10 mM
Non-proteins	
All-trans retinoic acid	25 ng/ml
alpha-tocopherol phosphate	3 ng/ml
para-Aminobenzoic acid	12 ng/ml
Ascorbic acid	12 ng/ml
Cholesterol	50 ng/ml
Choline chloride	3.5 µg/ml
17-beta Estradiol	0.5 pg/ml
Folic acid	0.33 µg/ml
Glutamine	2 mM
Glutathione	12 ng/ml
Hydrocortisone	0.25 ng/ml
Hypoxanthine Na	1.75 µg/ml
I-inositol	4.5 µg/ml
Linoleic acid	1 µg/ml
Lipoic acid	50 ng/ml
Non-essential amino acids	0.1 mM
O-Phosphoryl ethanolamine	5 µg/ml
Pyruvate Na	1 mM
Ribose	125 ng/ml
Selenous acid	8 ng/ml
Thiamine HCl	80 ng/ml
Triiodothyronine	0.2 pg/ml
Uracil	75 ng/ml
Vitamin B12	0.35 µg/ml
Xanthine	85 ng/ml
Proteins (human)	
Basic fibroblast growth factor	1 ng/ml
Collagen	100 ng/ml
Epidermal growth factor	10 ng/ml
Fetuin	40 ng/ml
Fibronectin	1.33 µg/ml
Insulin	2 µg/ml
Insulin-like growth factor 1	5 ng/ml
Laminin	20 ng/ml
Platelet-derived growth factor AA	2 ng/ml
Transferrin	50 µg/ml
Vitronectin	100 ng/ml
Human serum albumin	1.25 mg/ml

Conclusion

We present the composition of OUR defined medium without animal-derived products that can be used for routine culturing and in experimental settings for normal and cancer cells, *i.e.* OUR medium provides a leap towards a universal animal product-free cell culture medium. The formulation and preparation is available in our publication:

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