

Human Artificial Lymph Node Technology Platform

ProBioGen's human Artificial Lymph Node (HuALN) technology enables the prediction of human immune responses triggered by drug candidates *in vitro*. Using primary cells, this 3D-matrix bioreactor technology emulates *in vivo*-like reactions of a human lymph node to analyze e.g. immunogenicity, immune function and immunotoxicity of substances.

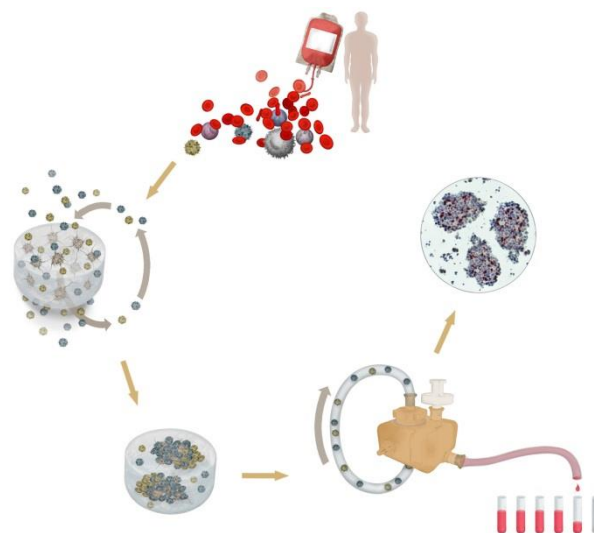
As modern biopharmaceuticals show a very high degree of species-specificity, animal models are inadequate to assess drug safety and drug efficacy. Drawbacks in the pharmaceutical arena during the last years have raised significant concerns about the predictability of animal models for immune function and immunotoxicity testing of biological therapeutics such as antibodies, glycoproteins, cytokines or vaccines in humans. The Human Artificial Lymph Node model (HuALN) is a bioreactor-based 3D-matrix technology for long-term cell cultivation to predict unwanted immunogenicity reactions in patients (e.g. neutralizing antibody formation, sensitization) of drug candidates early in drug development. In addition, it can also be used for efficacy assessment e.g. of vaccine candidates to select the most promising lead candidates.

HuALN Applications:

- Vaccines
- Proteins/Peptides
- Immune modulators
- Small molecules

The HuALN is operated with primary cells (PBMCs and PBMC-derived cells) from selected human donors (e.g. special HLA types). The cells are cultivated in a three-dimensional hydrogel matrix of a miniaturized, disposable bioreactor, which allows controlling wide ranges of cell feeding rates, gas supply and cell migration.

This ensures long-term cell cultivation and is the basis for cell migration and the formation of immune-competent micro-organoid structures (as opposed to simple multi-well-based PBMC assays in solution).



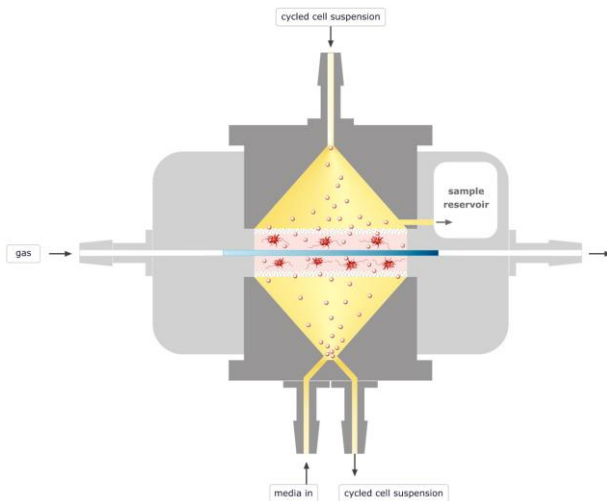
Advantages of the HuALN Model:

- 3D matrix-assisted culture model
- Formation of antigen-dependent immune competent micro-organoid structures
- Long-term culture (> 4 weeks)
- Continuous and moderate perfusion
- Daily sampling of culture supernatant for analytics (e.g. secreted cytokines)
- Long-term exposition of drug
- Multiple re-stimulations by APC and drug
- Broad panel of read-out parameters on T and B cell levels
- *In vitro* model, based on cells from humans with a fully trained immune system

The HuALN system supports both spreading and establishing of a stationary dendritic cell (DCs) network in the matrix, as well as T cell and B cell swarming and clustering within the DC network during bioreactor operation.

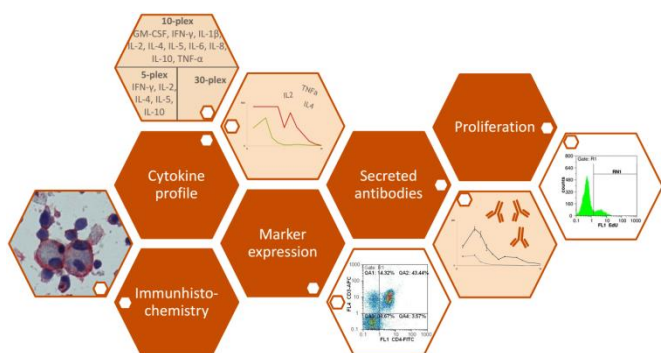
To support long-term cell performance cell culture media (and, optionally, a cell suspension)

perfuses the matrix with the embedded cells vertically, while gas for aeration passes the system horizontally.



The HuALN enables analyzing cellular and humoral immunity on the basis of various read-outs, e.g. cytokine profiles, antigen-specific antibodies, histological studies and cell characterization (e.g. by flow cytometry). After HuALN bioreactor operation, cells can be harvested and analyzed e.g. for cellular markers and in functional assays.

Depending on the substances tested, the bioreactor operation and analysis can be adjusted to focus on T cell or B cell responses, e.g. in analogy to animal experiments.



HuALN analytical parameters:

- Cytokine/Chemokine secretion
- Metabolics
- Histology
- Cellular analytics (Flow cytometry)
- Antibody secretion (IgM/IgG)

- Genomics and proteomics
- Microscopy/Imaging

ProBioGen has closed a number of collaborations on its proprietary HuALN model and related assays with pharmaceutical, biochemical and cosmetic companies, which are looking for reliable alternatives to animal-testing. This is increasingly pressing due to the E.U. ban of animal-testing for cosmetic substances, effective from 2013 on, for investigations on systemic toxicity, skin sensitization and allergy. In this context ProBioGen won the 26th Animal Protection Award by Germany's Federal Ministry of Food, Agriculture and Consumer Protection for its pioneering HuALN technology in 2007.

Literature:

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