

# Foundations for molecular and enzymatic functional surgery

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## **Abstract**

This paper presents an approach of molecular and enzymatic surgery for treatment of human diseases, including opportunity for use of systemic biology methods in planning of surgical interventions, possible biological components of a “molecular scalpel”, and problems of standardization, medical ethics and clinical trials of the new pharma-surgical toolbox. In conclusions is proposed to consider of molecular and enzymatic surgery methods as realization of the principles of “functional surgery” and also further development of fast track surgery with attaining the modern concept of a personalized approach to surgical treatment of the patient.

***Keywords:** engineering biology, enzymatic surgery, molecular surgery, synthetic biology, systems biology.*

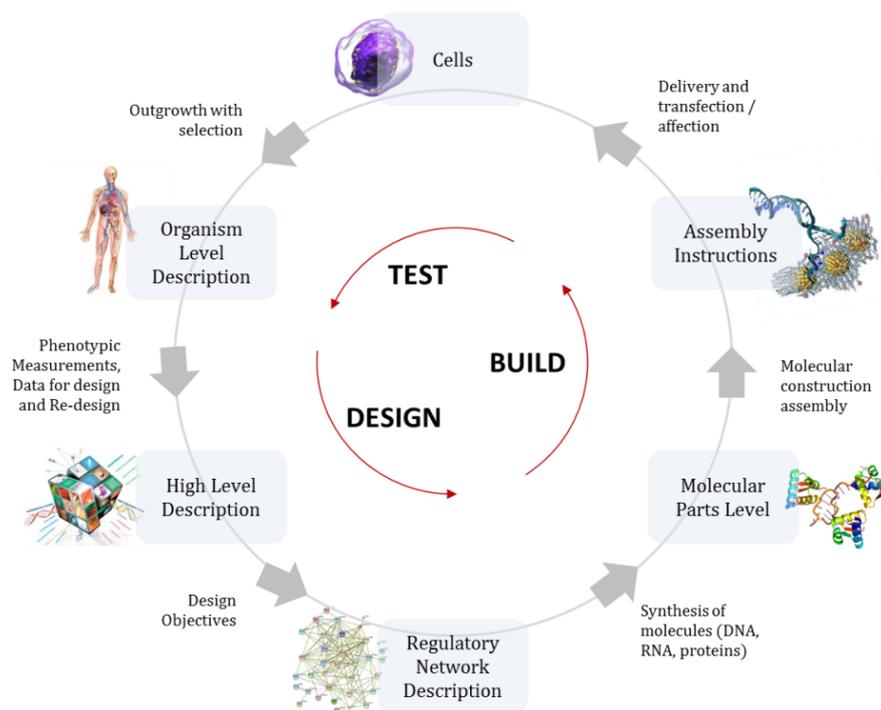
## **INTRODUCTION**

Surgical principles, united by the term “functional surgery” imply the performance of organ-preserving surgeries, often minimally invasive and aimed at correcting the body's systems while maintaining anatomy and restoring normal functions. In the XX century laparoscopic techniques, robotic assisted operations, Fast Track Surgery and Enhanced Recovery After Surgery (ERAS) concepts, etc. are exemplified the implementation of this principles. Modern molecular biology and biophysics expand these examples to perform functional operations at the molecular level [1].

Synthetic biology is an emerging field at the interface between biology and engineering, which has generated many expectations for beneficial biomedical and biotechnological applications [2]. However, the synthetic biology approach involves repetition or combination of existing biological solutions (recombinant proteins, BioBrick's parts, etc.). Engineering biology approach can be used to manipulate bioinformatics data and molecules for construct living systems to process chemicals, produce energy, provide food, and help maintain or enhance human health and our environment [3].

Engineering biology approach allows to present of molecular surgery's targets and tools as a united multilevel system (Pic. 1). Earlier developed toolbox models like TASBE (Tool-Chain to

Accelerate Synthetic Biological Engineering) [4], ATPG algorithms for cancer therapy [5], etc. are the cornerstone of this approach.



*Pic. 1. Multilayer model of molecular interventions in living organisms with engineering tools.*

The use an approach of systems and synthetic (engineering) biology allows to implement the advanced bioengineering concepts for “synthetic morphogenesis” [6] and “organ bud” [7], as well as toolbox for molecular and enzymatic surgery.

### **SURGERY ON MOLECULAR LEVEL**

The idea of surgery at the molecular level was first put forward by Nobel laureate Richard Feynman in 1959 [8] as an example of the potential use of nanoscale mechanisms for medical purposes. Further the concept of interventions at the molecular and tissue levels for changing the phenotype of tissues received its instrumental solution in the form of genetic engineering tools.

The term “molecular surgery” was first formulated in 1966 to describe the intervention on cell activity at the DNA level [9]. Further terminology has gained development in the concept of systems of genome editing (“surgery of genes”) [10], molecular surgery of cancer [11], etc.

Recently developed genome editing systems (based on CRISPR/Cas9, TALEN, ZFN) for therapeutic purposes allow to restore/recreate the normal cellular phenotype and, as a consequence, the normal functionality of pathologically altered tissues. Today the systems of molecular surgery for the treatment of cardiomyopathies, sickle-cell anemia and oncological diseases are in clinical

studies. The use of these methods for therapy of early fatal illness (Table 1) is extremely progressive.

*Table 1.* Examples of target diseases for molecular surgery therapy.

Disease	Target	Theoretical mechanism of action
Primary sclerosing cholangitis	More than 33 loci	Genetic improvement of genome mutations in human epithelial cells.
Cystic fibrosis	CFTR gene	Genetic improvement of CFTR gene in somatic cells.
Duchenne muscle dystrophy	Dystrophin gene	Genetic improvement or replacement of Dystrophin gene in somatic cells.
Leber's hereditary optic neuropathy	mtDNA mutations	Genetic improvement of mtDNA mutations.
Gallbladder diseases	Regulation of cholangiocytes transcriptomics / biliary microbiome engineering	Correction of secretion functions of cholangiocytes and/or biliary microbiota interventions.

The use of molecular surgery methods does possible treatment of genetic diseases (genome level), diseases associated with pathological regulation of genes (transcriptome level), diseases associated with pathological proteoforms of proteins (proteome level), diseases associated with the noise in genetic networks (epigenome level) and allows for interventions in prenatal and postnatal period (incl. adults).

## **ENZYMATIC SURGERY**

Correction of large-scale tissue defects is the goal of another discipline – an “enzymatic surgery”. The term “enzymatic surgery” was first formulated in 1981 to describe processes of DNA repair by special enzymes [12], but further the use of this methods has extended on manipulation with cells and tissues for example as a new treatment modality for burns [13]. Although today enzymes are mainly used for the treatment of digestive diseases, but the use of specific delivery systems allows for large-scale interventions to remodel pathologically altered tissues, for example, by delivering metalloproteinases to destroy proliferating fibrous tissue. The development of the enzymatic surgery is associated with selection of high-specific delivery vectors (cells, monoclonal antibodies, single-chain antibodies and fragments thereof), but also with the withdrawal and deactivation of toxic products and their utilization with the patient’s own organs (liver, gastrointestinal tract, kidneys, lungs, glands, etc.).

The good example of prototype of enzymatic surgery agent are the nanoparticles with biocomputing capabilities could potentially be used to create sophisticated autonomous nanodevices on DNA/RNA-based computing techniques [14].

*Table 2.* Examples of target diseases for enzymatic surgical therapy.

Disease	Target	Theoretical mechanism of action
Hepatic cirrhosis	Connective tissue	Local destruction of connective tissue components in liver.
Retinal detachment	Retina's cellular environment	Prevention of retinal detachment by local inhibition of vessel growth.
Down syndrome	Copy of the 21st chromosome	Destruction or inactivation of 3rd copy of the 21st chromosome in all somatic cells or only stem cells in human body.
Appendicitis	Gut microbiota components	Suppression of biological activity of microbiota-induced inflammation factors (both molecules and cells).

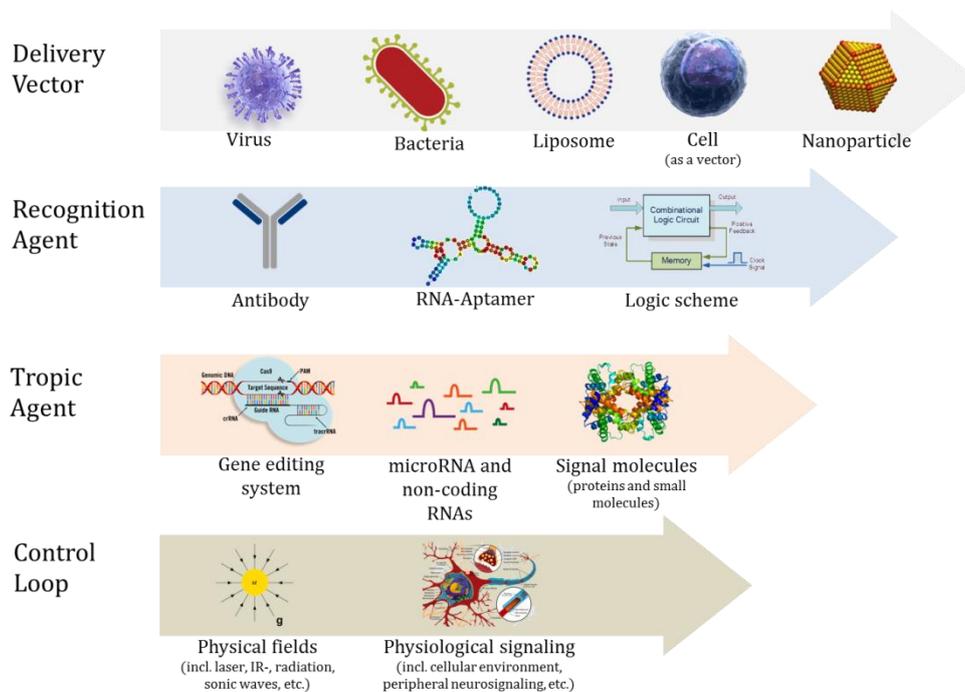
A correction of the spatial organization of enzymatic agents in a human body and an adjustment of physiological influence are required an external control facilities using of physical fields by the operator-surgeon (acoustic impact and electromagnetic, laser, infrared radiation, etc.). It seems advisable to development of an enzymatic toolbox for both functional and plastic surgery (proliferation under control of enzymatic agents and otherwise).

### **MOLECULAR TOOLBOX : A NEW PRECISION SCALPEL**

The effectiveness and specificity of systems of molecular and enzymatic surgery are associated with the improvement of delivery vectors. Highly specific delivery to target tissues can be carried out through cell-based vectors, viral systems (AAV, HIV, HSV, etc.), RNA-protein complexes and bactofection agents.

The use of gene therapy opportunities has allowed a molecular surgery of cancer diseases [15] and in 1992 the first time are discussed with FDA a permission of virus therapy for treatment of inoperable glial tumors. The reconciliation processes continued until 2015, when the FDA first approved therapy using oncolytic viruses [16].

Now the concept of toolbox of a “molecular scalpel” can be defined as conjugates with biological molecules (proteins and DNA/RNA conformations), cells, nanoparticles and control loops through physical fields (laser, infrared radiation, sonic waves, etc.) and physiological signaling (cellular environment, transcriptomic and metabolomic patterns, peripheral neurosignaling, etc.) (Pic. 2).



*Pic. 2.* Toolbox of the “molecular scalpel” components.

With regard to the traumatic nature of large-scale minimally invasive molecular operations, it can be assumed that recovery processes of this will be faster than in modern Fast Track Surgery techniques [17]. The key roles of “surgical team 2.0” will be an bioengineer with an operator-surgeon (as well as in robotic assisted operations) [18].

Previously the term “personalized surgery” was used exclusively to cancer therapy based on genetics data from patient [19]. Using a combination of coding (DNA, RNA) and signal (proteins and nucleic acids) molecules to regulate the body's functional for editing the genome and changing the cellular organization allows us to consider the possibility of personalizing surgical interventions based on the “omics” data of the patient's body (genome, transcript, metabolite, epigenome) to achieve an individual physiological response.

### **SAFETY, TRIAL SETS AND STANDARDIZATION**

At the furtherance of goal of clinical use of new therapeutic methods to the forefront there are problems of safety, clinical trials and tools standardization.

Today the problem of safety of use of bioengineering decisions is considered through creation of biological capabilities that enable the safe pursuit of advanced gene editing applications and protect against potential engineered genetic threats. It can be reached by control of gene editing, countermeasures and prophylactics, genetic remediation [20]. These measures have only a theoretical character and still wait for the realization in future.

Clinical trials and training of “surgical team 2.0” will differ from the corresponding procedures when using of existing drugs and medical products. The tissue-tropic of the used agents will demand development of adequate physiological models (for example, based on humanized animals) [21] and creation of life-like stands for surgical skills training [22]. The problem of clinical interpretation of multi-OMICS data is common of the training of medicals and is discussed now [23].

Problems with standardization can be considered both methods for unification of bioconstruction set/kit elements [24] and through standardized practice procedures like a Good Laboratory (GLP), Manufacturing (GMP) and Tissue (GTP) Practices, including for standardized manufacturing of viral [25] and cellular products [26]. Formalization and standardization those mechanisms are devoted an amount of initiatives in the form of consortiums, organizations, committees and working groups (Tab. 3).

Table 3. Initiatives for Advanced Therapies Standardization.

Initiative	Description	Ref.
Standards Coordinating Body For Cellular/Gene and Regenerative Therapies and Cell-Based Drug Discovery (USA, 2016)	Promoting the development of new standards in manufacturing and processing and coordinating international standard-development efforts; formulation of global regulatory convergence initiatives; clarification of the surrounding regulatory treatment of different human cell and tissue products by FDA; initiatives to improve existing regulatory pathways to support rapid evaluation of regenerative medicine and cell and gene therapy products.	[27]
Synthetic Biology Open Language Developer’s Group (USA, 2011)	The Synthetic Biology Open Language (SBOL) can be used to represent genetic designs through a standardized vocabulary of schematic glyphs (SBOL Visual) as well as a standardized digital format (SBOL Data).	[28]
Protein Capture Reagents Program (USA, 2006)	NIH program for producing of standardized binding reagents for research uses, including recombinant monoclonal antibodies, recombinant antibodies, aptamers (nucleic-acid-based reagent), non-antibody binding reagents.	[29]
iGEM Foundation (Registry of Standard Biological Parts) (USA, 2002)	The iGEM Registry has over 20,000 documented parts for genetic construction engineering based on biobricks. The Catalog organizes many of these parts by part type, chassis, function, and more.	[30]
Good Laboratory and Manufacturing Practices Committees (WHO, 1967)	The establish of quality system of management controls to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of chemical (including pharmaceuticals) non-clinical safety tests (GLP), and quality control management of manufacturing (GMP) include hygiene, validation, self-inspection, personnel, premises, equipment, materials and documentation.	[31]
American Type Culture Collection – ATCC (USA, 1925)	The acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other materials for research and development. Collection contains of standard eukaryotic and prokaryotic cells, virus strains, etc. as research models and chassis (established, emerging and potential).	[32]

The problems with safety, trial sets and standardization of complex biomolecular systems are only at the initial stage of their solution.

### **ETHICAL ASPECTS**

It is important to solve of ethical problems for using of advanced technologies in a clinical practice. The use of the terms “genome modification” and “genetic engineering” as applied to invasive interventions at the molecular level does not fully relation the essence of the phenomenon. For example, the concept “gene modification” means a complex of the interventions that are differing by the physical and biological natures. The use of this term in relation to different objects can lead the ethics researcher to incorrect conclusions.

Recently fetal (ante-natal) surgical operations have spread [33]. Fetal surgery in-utero has been attempted for various congenital anomalies including congenital diaphragmatic hernia (CDH), spina bifida and urinary tract abnormalities, twin-twin transfusion syndrome, etc. [34].

The ethical aspects of the maternal-fetal surgery are considered within the framework of “the fetus as a patient” [35]. Has received worldwide fame the photo of Samuel Armas's tiny hand apparently grasping the finger of the perinatal surgeon [36] who was repairing the spine of the 21-week old fetus for spina bifida in 1999 [37].



*Pic. 3. “Hand of Hope”*: an example of ante-natal surgical patient with a successful outcome. 21-week-old Samuel Armas during surgical operations © Michael Clancy, 1999.

Tools for molecular surgery allows performing such operations at any age. The use of the terms “molecular scalpel” [38], “molecular surgery” for such interventions will also allow to avoid therapeutic misconception in a patient.

At the same time development and realization of an adequate animal models, a “life-like” stands for surgical skills training and an management of clinical sites wills represent a serious ethical problem which can be partially solved by using of systems of the “organ-on-chip” class [39] and clinical trials “in a dish” [40].

## CONCLUSIONS

Modern methods of performing minimally invasive surgical procedures to restore the normal functions of organs can be supplemented by the method of using molecular agents - enzymes, immuno-drugs, and other molecular machines. For another thing the molecular and enzymatic surgery approach can be the key to solving ethical problems of human genome engineering.

To date the prototype of molecular surgery systems undergo clinical trials for the treatment of some diseases, and prospective areas of their using is the therapy of diseases and conditions previously available for only invasive therapy or incurable at all. There is an unimaginable amount of unsolved problems in the enhancing of human [41], where there is a place for these methods.

High-tech implementation of the principles of functional molecular and enzymatic surgery in the forms of genome editing systems and theranostic agents (providing both diagnostics and treatment) represent the advanced methodical method of “physiological surgery” by Ivan Pavlov (1902) [42] and attaining the modern concept of a personalized approach to surgical treatment of the patient.

## References

- [1] I. D. Klabukov, P. Y. Volchkov, A. V. Lyundup, and T. G. Dyuzheva, “Molecular and enzymatic functional surgery of the future,” in *Proceedings of National Surgical Congress 2017 jointly with XX Jubilee Congress of the Society of Endoscopic Surgeons of Russia (SESR)*, 2017, p. 1794.
- [2] D. Gregorowius and A. Deplazes-Zemp, “Societal impact of synthetic biology: responsible research and innovation (RRI),” *Essays Biochem.*, vol. 60, no. 4, pp. 371–379, 2016.
- [3] D. Endy, “Foundations for engineering biology,” *Nature*, vol. 438, no. 7067, pp. 449–453, Nov. 2005.
- [4] J. Beal, R. Weiss, D. Densmore, a. Adler, J. Babb, and S. Bhatia, “TASBE: A tool-chain to accelerate synthetic biological engineering,” *Proc. 3rd Int. Work. Bio-Design Autom.*, vol. 2, pp. 19–21, 2011.
- [5] P.-C. K. Lin and S. P. Khatri, “ATPG for Cancer Therapy,” in *Logic Synthesis for Genetic Diseases*, New York, NY: Springer New York, 2014, pp. 77–92.
- [6] B. P. Teague, P. Guye, and R. Weiss, “Synthetic Morphogenesis,” *Cold Spring Harb. Perspect. Biol.*, vol. 8, no. 9, p. a023929, Sep. 2016.
- [7] N. Rusk, “Reproducibly generating organ buds in vitro.,” *Nat. Methods*, vol. 12, no. 7, p. 600, Jul. 2015.
- [8] R. P. Feynman, “There’s plenty of room at the bottom,” *Eng. Sci.*, vol. 23, no. 5, pp. 22–36, 1960.
- [9] R. G. Denkewalter and M. Tishler, “Drug Research — Whence and Whither,” in *Fortschritte der Arzneimittelforschung / Progress in Drug Research / Progrès des recherches pharmaceutiques*, Basel: Birkhäuser Basel, 1966, pp. 11–31.
- [10] B. Hobom, “[Surgery of genes – at the doorstep of synthetic biology],” *Med. Klin.*, vol. 75, no. 24, pp. 14–21, 1980.
- [11] J. A. Roth, “MOLECULAR SURGERY FOR CANCER,” *Arch. Surg.*, vol. 127, no. 11, pp. 1298–1302, 1992.
- [12] M. C. Paterson, N. T. Bech-Hansen, and P. J. Smith, “Heritable Radiosensitive and DNA Repair-Deficient Disorders in Man,” in *Chromosome Damage and Repair*, Boston, MA: Springer US, 1981, pp. 335–354.
- [13] L. Rosenberg, O. Lapid, A. Bogdanov-Berezovsky, et al., “Safety and efficacy of a proteolytic enzyme for

enzymatic burn debridement: a preliminary report.," *Burns*, vol. 30, no. 8, pp. 843–50, Dec. 2004.

- [14] M. P. Nikitin, V. O. Shipunova, S. M. Deyev, and P. I. Nikitin, "Biocomputing based on particle disassembly," *Nat. Nanotechnol.*, vol. 9, no. 9, pp. 716–722, Aug. 2014.
- [15] R. Stone, "Molecular 'surgery' for brain tumors," *Science (80-. )*, vol. 256, no. 5063, pp. 1513–1513, Jun. 1992.
- [16] E. Dolgin, "Oncolytic viruses get a boost with first FDA-approval recommendation.," *Nat. Rev. Drug Discov.*, vol. 14, no. 6, pp. 369–71, Jun. 2015.
- [17] H. Kehlet, "Fast-track surgery - An update on physiological care principles to enhance recovery," *Langenbeck's Archives of Surgery*, vol. 396, no. 5, pp. 585–590, 2011.
- [18] H. Azimian, "Surgical Robotic Tools," in *Bioengineering for Surgery: The Critical Engineer Surgeon Interface*, 2016 by H. Azimian. Published by Elsevier Ltd. All rights reserved, 2015, pp. 91–112.
- [19] J. Yu, S.-H. he Liu, R. Sanchez, J. Nemunaitis, E. Rozengurt, and F. C. Brunicardi, "Pancreatic cancer actionable genes in precision medicine and personalized surgery," *Surgeon*, 28-Jun-2016.
- [20] R. D. Wegrzyn, "Safe Genes," Washington, DC, 2016.
- [21] B. Delire, P. Stärkel, and I. Leclercq, "Animal Models for Fibrotic Liver Diseases: What We Have, What We Need, and What Is under Development.," *J. Clin. Transl. Hepatol.*, vol. 3, no. 1, pp. 53–66, Mar. 2015.
- [22] J. Kane, N. Reyes, M. Minneti, P. Talving, W. Schooler, W. Garner, and J. Carey, "High Fidelity Surgical Simulation Using Perfused Fresh Cadaveric Tissue: A Life-Like Model for Surgical Training," *Plast. Reconstr. Surg.*, vol. 130, p. 59, Jul. 2012.
- [23] N. Chinai, F. Bintcliffe, E. M. Armstrong, J. Teape, B. M. Jones, and K. B. Hosie, "Does every patient need to be discussed at a multidisciplinary team meeting?," *Clin. Radiol.*, vol. 68, no. 8, pp. 780–784, 2013.
- [24] K. M. Müller and K. M. Arndt, "Standardization in Synthetic Biology," in *Methods in molecular biology (Clifton, N.J.)*, vol. 813, Humana Press, 2012, pp. 23–43.
- [25] M. Lusky, "Good Manufacturing Practice Production of Adenoviral Vectors for Clinical Trials," *Hum. Gene Ther.*, vol. 16, no. 3, pp. 281–291, Mar. 2005.
- [26] P. Wuchter, K. Bieback, H. Schrezenmeier, M. Bornhäuser, L. P. Müller, H. Bönig, W. Wagner, R. Meisel, P. Pavel, T. Tonn, P. Lang, I. Müller, M. Renner, G. Malcherek, R. Saffrich, E. C. Buss, P. Horn, M. Rojewski, A. Schmitt, A. D. Ho, R. Sanzenbacher, and M. Schmitt, "Standardization of Good Manufacturing Practice-compliant production of bone marrow-derived human mesenchymal stromal cells for immunotherapeutic applications," *Cytotherapy*, vol. 17, no. 2, pp. 128–139, 2015.
- [27] T. Hayakawa, I. Harris, J. Joung, N. Kanai, S. Kawamata, S. Kellathur, J. Koga, Y.-C. Lin, Y. Maruyama, J. McBlane, T. Nishimura, M. Renner, A. Ridgway, P. Salmikangas, N. Sakamoto, D. Sato, Y. Sato, Y. Toda, A. Umezawa, M. Werner, and S. Wicks, "Report of the International Regulatory Forum on Human Cell Therapy and Gene Therapy Products," *Biologicals*, vol. 44, no. 5, pp. 467–479, 2016.
- [28] J. L. Contreras, A. K. Rai, and A. W. Torrance, "Intellectual property issues and synthetic biology standards.," *Nat. Biotechnol.*, vol. 33, no. 1, pp. 24–5, Jan. 2015.
- [29] A. Bradbury and A. Plückthun, "Reproducibility: Standardize antibodies used in research.," *Nature*, vol. 518, no. 7537, pp. 27–9, Feb. 2015.
- [30] R. P. Shetty, D. Endy, and T. F. Knight, "Engineering BioBrick vectors from BioBrick parts.," *J. Biol. Eng.*, vol. 2, no. 1, p. 5, Apr. 2008.
- [31] "WHO good manufacturing practices for pharmaceutical products," in *WHO Technical Report Series*, no. 961, Geneva: World Health Organization, 2011, pp. 94–147.
- [32] G. Stacey, A. Doyle, R. Hay, R. Johnson, J. Beck, R. MacLeod, D. Fritze, B. Bolton, B. Parodi, O. Aresu, P. Visconti, M. Cesaro, R. Lorenzini, T. Ruzzon, M. Takeuchi, H. Mizusawa, H. Tanabe, T. Masui, T. Sofuni, and T. Ohno, "Cell Banks: A Service to Animal Cell Technology," in *Encyclopedia of Cell Technology*, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2003.

- [33] N. S. Adzick, "Fetal surgery for spina bifida: past, present, future.," *Semin. Pediatr. Surg.*, vol. 22, no. 1, pp. 10–7, Feb. 2013.
- [34] P. Sala, F. Prefumo, D. Pastorino, D. Buffi, C. R. Gaggero, M. Foppiano, and P. De Biasio, "Fetal surgery: an overview.," *Obstet. Gynecol. Surv.*, vol. 69, no. 4, pp. 218–28, Apr. 2014.
- [35] F. A. Chervenak and L. B. McCullough, "Ethics of maternal-fetal surgery," *Semin. Fetal Neonatal Med.*, vol. 12, no. 6, pp. 426–431, 2007.
- [36] T. S. Collett, "Previability Abortion and the Pain of the Unborn," *Wash. Lee Law Rev.*, vol. 71, no. 2, pp. 1211–1231, 2014.
- [37] N. Tulipan and J. P. Bruner, "Fetal surgery for spina bifida.," *Lancet (London, England)*, vol. 353, no. 9150, p. 406; author reply 407, Jan. 1999.
- [38] B. Hobom, "[With the scalpels of gene surgery against disease and hunger].," *Ther. Ggw.*, vol. 119, no. 2, pp. 125–38, Feb. 1980.
- [39] C. Luni, E. Serena, and N. Elvassore, "Human-on-chip for therapy development and fundamental science.," *Curr. Opin. Biotechnol.*, vol. 25, pp. 45–50, Feb. 2014.
- [40] D. G. Strauss and K. Blinova, "Clinical Trials in a Dish," *Trends Pharmacol. Sci.*, vol. 38, no. 1, pp. 4–7, Jan. 2017.
- [41] I. Klabukov, "Engineering Biology Problems Book," *SSRN Electron. J.*, p. 54, 2016. doi:10.2139/ssrn.2898429
- [42] J. P. Pawlow, "Die physiologische Chirurgie des Verdauungskanal," *Ergebnisse der Physiol.*, vol. 1, no. 1, pp. 246–284, 1902.