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Bioorthogonal chemistry in the reproductive medicine

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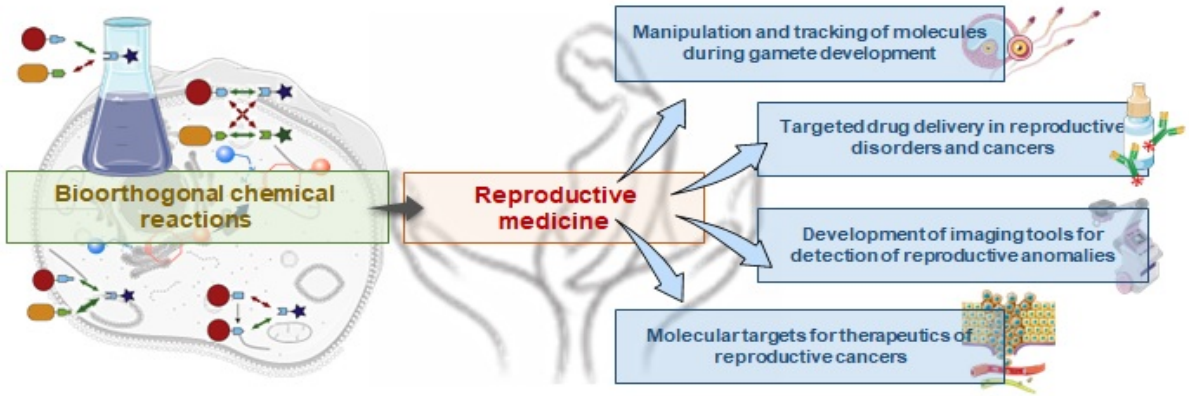
Editorial

ABSTRACT



The expanding field of bioorthogonal chemistry has demonstrated significant potential in advancing reproductive medicine. This comprehensive review elucidates the multifaceted applications of bioorthogonal chemistry across various aspects of reproductive medicine, including gamete biology, energetics and metabolic regulations of gametes, targeted drug delivery, detection and therapeutic of endometriosis and polycystic ovarian syndrome (PCOS), developments of diagnostic tools and new management approaches to reproductive cancers. In gamete biology, bioorthogonal reactions enable the precise manipulation and tracking of biomolecules within gametes, thus facilitating a deeper understanding of gamete development, maturation, and interaction. Bioorthogonal chemistry also plays an indispensable role in deciphering the intricate energetics and metabolic regulations governing gamete function and competence, consequently fostering the development of novel therapeutic interventions. Targeted drug delivery, utilizing bioorthogonal click chemistry, can improve the specificity and efficacy of pharmacological treatments in reproductive disorders, such as endometriosis and PCOS. In the realm of reproductive diagnostics, bioorthogonal chemistry engenders innovative tools for sensitive and noninvasive detection of reproductive anomalies. Finally, the integration of bioorthogonal strategies in studying reproductive cancers can uncover new molecular targets for therapeutics, leading to more effective treatment modalities. Collectively, this review highlights the paramount importance of bioorthogonal chemistry in revolutionizing reproductive medicine and fostering breakthroughs in the comprehension and management of reproductive health.

Keywords: biorthogonal chemistry, fertilization, gamete biology, reproductive medicine

INTRODUCTION

The burgeoning field of reproductive medicine has seen an upsurge in the application of advanced chemical and biological techniques to address fertility issues and enhance the understanding of the mechanisms underlying reproduction. One such nascent approach that has been increasingly employed is bioorthogonal chemistry, a discipline that exploits non-canonical, biologically inert reactions to facilitate the study of complex biological systems.[1] Owing to its non-perturbative nature, bioorthogonal chemistry enables the real-time interrogation of reproductive biomolecules and their spatiotemporal dynamics with minimal interference in their native *milieu*.[2] This discussion delineates the relevance of bioorthogonal chemistry in reproductive medicine and underscores its capacity to further our knowledge of the intricate biological phenomena governing human fertility.

Bioorthogonal reactions are characterized by their orthogonality, selectivity, and rapid kinetics, which imbue them with the ability to operate unobtrusively in living systems.[3] These traits render bioorthogonal chemistry an invaluable tool for studying reproductive processes, such as gametogenesis,

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fertilization, and embryo development, which are governed by delicate molecular interplays.[3,4] By employing novel chemical reporters and bioorthogonal ligation strategies, researchers have been able to elucidate the roles of specific biomolecules, including proteins, nucleic acids, and lipids, in reproductive processes and pathologies.[5] A salient example of the implementation of bioorthogonal chemistry in reproductive medicine lies in the investigation of post-translational modifications (PTMs) and their consequences on cellular signaling pathways.[6] Through the integration of bioorthogonal techniques, researchers can selectively label and monitor PTMs *in situ*, providing insights into their regulatory functions in the context of reproductive biology. Furthermore, bioorthogonal chemistry has been harnessed to study the dynamics of sperm-egg interactions, revealing novel molecular players and spatiotemporal cues that orchestrate successful fertilization.[7]

In addition to its contributions to fundamental research, bioorthogonal chemistry holds promise for the development of novel diagnostic and therapeutic strategies in reproductive medicine.[8] For instance, by leveraging bioorthogonal approaches to selectively target and modulate the activity of reproductive biomolecules, scientists are working towards the design of innovative fertility treatments and contraceptive modalities.[9] Moreover, the linkage of bioorthogonal chemistry and advanced imaging techniques has enabled the non-invasive visualization of reproductive processes, paving the way for improved diagnostic tools and personalized medicine.[10] The advent of bioorthogonal chemistry has precipitated a paradigm shift in the study and manipulation of reproductive processes, fostering a more comprehensive understanding of the molecular underpinnings of fertility and offering new avenues for therapeutic intervention.

Bioorthogonal Chemistry in Gamete Biology

Gamete biology is an area of interest in reproductive medicine where bioorthogonal chemistry is being increasingly used. Bioorthogonal chemistry can be used to label specific molecules within gametes, such as proteins or lipids, which can then be visualized and tracked within living systems.[11] This allows researchers to study the dynamics of gamete development, maturation, and fertilization, and to identify potential targets for therapeutic interventions. Innovative bioorthogonal tools, like bioorthogonal proteomics, have been employed to unveil the spatiotemporal proteome dynamics in gametes and embryos, revealing intricate regulatory networks governing gamete maturation and fertilization competency.[12] For example, recent studies have used bioorthogonal chemistry to label glycosylated proteins within sperm, which are thought to play a role in sperm-egg interactions during fertilization.[13] By tracking the movement of these proteins within living sperm, researchers have been able to gain new insights into the mechanisms underlying fertilization,[8] and to identify potential targets for drugs that could enhance fertility or prevent fertilization altogether. Bioorthogonal chemistry thus offers a plethora of opportunities for dissecting the molecular intricacies of gamete biology, paving the way for the development of novel therapeutic strategies to address infertility and improve reproductive health.

Energetics and Metabolic Regulations of Gametes

In the intricate and dynamic landscape of reproductive biology, energy management and metabolic regulation play a paramount role in the maintenance of healthy sperm and ova production. The orchestration of these processes encompasses the fine-tuning of various metabolic pathways, redox homeostasis, and cellular signaling, all of which are critical for the optimal function and survival of germ cells.[14] As a testament to the ever-evolving scientific inquiry, the discussion delves into the molecular underpinnings of these phenomena, shedding light on the metabolic interactions involved and the potential for manipulation and control using cutting-edge bioorthogonal chemistry reagents and reactions.

Germ cell development and maturation necessitate a tightly regulated metabolic microenvironment, which is chiefly governed by the interplay between glycolysis, oxidative phosphorylation, and the Krebs cycle.[14] The energy derived from these catabolic processes ensures the preservation of genomic integrity, the establishment of epigenetic modifications, and the formation of intricate membrane structures in sperm and ova.[14] Consequently, perturbations in this metabolic *milieu* can have profound ramifications on fertility outcomes.

A crucial component of this metabolic regulation is the delicate balance of reactive oxygen species (ROS) production and scavenging.[14] The redox equilibrium is vital for the modulation of cellular signaling pathways, such as those orchestrated by protein kinases, phosphatases, and transcription factors, which are indispensable for germ cell maturation and function. Dysregulation of redox homeostasis can result in oxidative stress (OS), contributing to sperm dysfunction and oocyte degeneration, thereby compromising fertility.[15] Recent advances in bioorthogonal chemistry have paved the way for the development of novel reagents and reactions that offer unprecedented precision in the interrogation and manipulation of biological processes.[10] These tools hold immense promise for unveiling the complex metabolic and signaling networks governing sperm and ova health, as well as for devising targeted therapeutic interventions to ameliorate fertility issues.[2] By harnessing the power of bioorthogonal chemistry, researchers can selectively modulate key metabolic and redox pathways, thereby offering new avenues for the optimization of germ cell energy management and control. Thus, the exploration of energy management and metabolic regulation in the context of healthy sperm and ova production serves as a fertile ground for scientific investigation. The synergistic application of bioorthogonal chemistry reagents and reactions in this arena holds tremendous potential for elucidating the intricacies of germ cell biology and devising innovative strategies for fertility enhancement and preservation.

Targeted Drug Delivery in Reproductive Medicine

Another area where bioorthogonal chemistry can be used in reproductive medicine is in the development of targeted drug

delivery systems.[16] Traditional drug delivery methods often result in non-specific targeting of tissues, which can lead to unwanted side effects and reduced efficacy.[17] By using bioorthogonal chemistry to target specific molecules within cells or tissues, researchers can design drug delivery systems that are more precise and effective. Bioorthogonal reactions involve the selective reaction of small molecules or chemical groups with other molecules to form stable covalent bonds. Bioorthogonal reactions can be used to attach drugs to specific molecules, such as antibodies or peptides, which can then be used to target specific cells or tissues.[5] The preeminent utilities of bioorthogonal chemistry in reproductive medicine involve the spatiotemporal control of drug release, enhanced tissue targeting, and ameliorated bioavailability of therapeutics.[18] In reproductive medicine, bioorthogonal chemistry can also be used to label specific proteins or receptors on the surface of cancer cells, which can then be targeted by drug-loaded nanoparticles or other delivery vehicles. This approach has the potential to improve the efficacy of chemotherapy while reducing the risk of side effects.[18] The advent of bioorthogonal chemistry engenders a new era in reproductive medicine, as it facilitates the development of innovative strategies for precise drug delivery. Utilizing the quintessential attributes of bioorthogonal reactions, such as the copper-free click chemistry and Staudinger ligation, researchers are now able to devise personalized treatments for an array of reproductive disorders, ranging from endometriosis to polycystic ovary syndrome (PCOS).[18]

Endometriosis and Polycystic Ovarian Syndrome

Bioorthogonal chemistry can be used to target specific cells within the reproductive system, such as the endometrial cells lining the uterus. This could be useful in the treatment of endometriosis, a chronic gynecological disorder marked by aberrant ectopic growth of endometrial tissue which can cause pain and infertility. In endometriosis, the application of bioorthogonal chemistry has facilitated an enhanced understanding of intricate cellular and molecular mechanisms, such as aberrant proteostasis, inflammatory signaling, and angiogenic processes.[11] Through the exploitation of bioorthogonal click chemistry, researchers have successfully tagged and traced post-translational modifications (PTMs) in endometrial cells, shedding light on dysregulated signaling pathways.[11] By developing drug delivery systems that target these cells specifically, researchers could develop more effective treatments with fewer side effects. By leveraging cutting-edge bioorthogonal strategies, the scientific community continues to unravel the multifaceted pathophysiology of endometriosis, paving the way for innovative diagnostic and therapeutic modalities.

In recent years, its application in deciphering the molecular underpinnings of polycystic ovary syndrome (PCOS), a heterogeneous endocrine disorder affecting approximately 5-10% of women of reproductive age, has garnered significant attention. Utilizing bioorthogonal chemistry, researchers have elucidated key insights into the interplay between complex biochemical signaling networks and cellular processes underlying PCOS.[19] By exploiting the bioorthogonal click chemistry approach, scientists have successfully incorporated noncanonical amino acids and bioorthogonal functional groups into endogenous proteins *in vivo*. This has facilitated the selective visualization and tracking of discrete protein populations in ovarian granulosa cells and theca cells, revealing novel protein-protein interactions and post-translational modifications associated with PCOS.[19] Moreover, bioorthogonal chemistry has been instrumental in the development of targeted therapeutics. Incorporation of bioorthogonal moieties into small molecule inhibitors has facilitated their selective delivery to the aberrant signaling proteins implicated in PCOS, thereby circumventing off-target effects.

Diagnostic Tools in Reproductive Medicine

Bioorthogonal chemistry is also being used in the development of diagnostic tools for reproductive medicine. Imaging techniques are essential for diagnosing various reproductive disorders. Traditional imaging techniques, such as X-rays and ultrasound, have limitations in terms of sensitivity and specificity. Bioorthogonal chemistry has enabled the development of new imaging techniques that are more sensitive and specific. For example, bioorthogonal chemistry has enabled the development of imaging techniques based on click chemistry.[20] Click chemistry involves the selective reaction of small molecules or chemical groups with other molecules to form stable covalent bonds. Click chemistry has been used to develop imaging probes that can selectively label specific molecules in biological systems. These probes can be used to visualize various cellular processes, including those involved in reproduction.[20] For example, bioorthogonal chemistry can be used to label specific proteins or biomarkers within the reproductive system, which can then be detected using imaging techniques such as magnetic resonance imaging (MRI) or positron emission tomography (PET). This approach has the potential to improve the accuracy of diagnoses and to allow for earlier detection of reproductive conditions. For example, in the diagnosis of ovarian cancer, bioorthogonal chemistry could be used to label specific proteins or biomarkers within the ovaries, which could then be detected using MRI or PET. This could allow for earlier diagnosis and more effective treatment. Traditional techniques for studying reproductive biology, such as Western blotting and immunofluorescence, have limitations in terms of sensitivity and specificity. Bioorthogonal chemistry has enabled the development of new techniques for studying reproductive biology based on click chemistry. Click chemistry has been used to develop probes that can selectively label specific molecules in biological systems. These probes can be used to study various cellular processes, including those involved in reproduction, with high sensitivity and specificity. For instance, bioorthogonal chemistry has been used to develop techniques for studying protein interactions in live cells. These techniques involve the introduction of chemical groups into specific proteins that can then react with specific chemical probes through a bioorthogonal reaction. The interaction between the proteins can be visualized

using fluorescence microscopy, enabling the study of protein-protein interactions in live cells.

BIOORTHOGONAL CHEMISTRY IN REPRODUCTIVE CANCERS

Reproductive cancers, such as ovarian, cervical, and prostate cancers, are some of the most common types of cancer, and they have a significant impact on morbidity and mortality rates. Bioorthogonal chemistry has been used to develop various imaging techniques that aid in the diagnosis of reproductive cancers.[21] One such technique is the use of clickable probes that selectively react with biomolecules present on cancer cells. For example, a clickable probe that selectively binds to the prostate-specific membrane antigen (PSMA) has been developed for the diagnosis of prostate cancer.[21] This probe can be labeled with a fluorophore or a radionuclide, allowing for the visualization of PSMA-expressing cells using fluorescence or positron emission tomography (PET) imaging. A study showed the feasibility of preparing PSMA-targeted MBs and the advantages of using bioorthogonal chemistry for targeted ultrasound probes. The study developed microbubbles (MBs) targeting PSMA using bioorthogonal chemistry.[22] Streptavidin-labeled MBs were treated with a biotinylated tetrazine (MBTz) and targeted to PSMA expressing cells using trans-cyclooctene (TCO)-functionalized anti-PSMA antibodies (TCO-anti-PSMA). In vitro experiments using a flow chamber showed increased binding of MBs to PSMA positive cells using both pretargeting and direct targeting approaches. *In vivo* experiments using a human xenograft tumor model showed increased ultrasound signal enhancements with PSMA-targeted MBs compared to non-targeted MBs. A head-to-head study using mice bearing both PSMA positive and negative tumors showed higher ultrasound signals with human PSMA expressing tumors.[22] Another application of bioorthogonal chemistry in cancer diagnosis is the use of metabolic labeling. This involves the incorporation of a non-natural amino acid into cancer cells, which can then be selectively labeled with a small molecule probe. For example, the incorporation of an azide-containing amino acid into ovarian cancer cells can be selectively labeled with a fluorophore-tagged alkyne. This allows for the visualization of cancer cells *in vivo* using fluorescence imaging.

Targeted drug delivery systems allow for the selective delivery of drugs to cancer cells, minimizing the side effects associated with traditional chemotherapy. One such system is the use of antibody-drug conjugates (ADCs) that selectively bind to cancer cells and release the drug payload upon internalization.[23] However, the conjugation of drugs to antibodies can be challenging, as it may interfere with the binding affinity of the antibody. Bioorthogonal chemistry can overcome this challenge by using bioorthogonal reactions to selectively conjugate drugs to antibodies. For example, the use of a genetically encoded unnatural amino acid that contains an alkyne group allows for the selective conjugation of drugs to antibodies using click chemistry. This allows for the creation of ADCs with improved pharmacokinetics and reduced toxicity. Another application of bioorthogonal chemistry in cancer therapy is the use of photodynamic therapy (PDT).23 PDT involves the activation of a photosensitizer in the presence of light, which generates reactive oxygen species that induce cell death. However, the activation of the photosensitizer can be challenging, as it requires specific wavelengths of light that may not penetrate deep into tissue. Bioorthogonal chemistry can overcome this challenge by using bioorthogonal reactions to activate the photosensitizer *in situ*. For example, the use of a bioorthogonal catalyst that selectively activates the photosensitizer in the presence of a bioorthogonal reagent can be used to generate reactive oxygen species in cancer cells. This allows for the selective destruction of cancer cells while minimizing damage to healthy tissue.

A groundbreaking investigation extended the application of bioorthogonal labeling methods to clinically relevant issues.[24] The study demonstrated the initial utilization of a glycoproteomic technique on cultured human tissues. The research involved the cultivation of both healthy and cancerous prostate tissues, which were sliced and grown with Ac4 ManNAz, a biosynthetic precursor of sialic acid containing azide functional groups.[25,26] This compound was transformed into azidosialic acid and incorporated into sialoglycoproteins on the surface of the cells and secreted by them. By using chemical biotinylation, followed by enrichment and mass spectrometry, researchers were able to identify elevated or uniquely expressed glycoproteins in cancerous prostate tissue. Utilizing bioorthogonal chemistry in combination with advanced imaging modalities, researchers have successfully visualized the dysregulated metabolic pathways and aberrant molecular interactions driving tumorigenesis in reproductive cancers. These insights facilitate the rational design of targeted therapeutics, which can be selectively delivered to malignant cells, minimizing off-target effects and circumventing chemoresistance. Further, as discussed above, bioorthogonal reactions can facilitate targeted drug release and activation within tumor microenvironments. For instance, bioorthogonal cleavage reactions, have been employed to uncage prodrugs within neoplastic tissue, thereby minimizing systemic toxicity.

CONCLUSION

Bioorthogonal chemistry has several applications in reproductive medicine, including the development of new imaging techniques, targeted drug delivery systems, techniques for studying reproductive biology and improve the accuracy of diagnoses. By using chemical reactions that occur within living systems without interfering with biological processes, researchers can develop new tools and approaches that are more precise and effective than traditional methods. As this field continues to evolve, it is likely that we will see many more exciting developments.

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